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(21) International Application Number: PCT/CA97/00828 (22) International Filing Date: 7 November 1997 (07.11.97) (30) Priority Data: 08/746,420 8 November 1996 (08.11.96) US (71) Applicant ( <i>for all designated States except US</i> ): NoAB IMMUNOASSAY INC. [CA/CA]; Unit #1, 50 Valleywood Drive, Markham, Ontario L3R 6E9 (CA). (72) Inventors; and (75) Inventors/Applicants ( <i>for US only</i> ): EWART, Thomas, G. [CA/CA]; 110 McClure Drive, King City, Ontario L7B 1B9 (CA). BOGLE, Gavin, T. [CA/CA]; Suite 406, 24 Wellesley Street, West, Toronto, Ontario M4Y 1G1 (CA). (74) Agent: WOODLEY, John, H.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
(54) Title: NANOPARTICLES BIOSENSOR			
(57) Abstract <p>Biosensor technology based on the labelling entities having particle reporters provides cost competitive readily manufactured assay devices. Submicron particles of uniform dimension in metals, polymers, glasses, ceramics and biological structures such as phages are used as the labelling entities. Such reporter particles greatly increase the sensitivity and accuracy, and provide a variety of assay techniques for determining analyte presence in a sample. The particles may have dielectric, paramagnetic and/or phosphorescent properties; such particles are particularly useful in a variety of competition type assays. Novel phosphor and phage particles are provided for use as unique labelling entities.</p>			

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# **NANOPARTICLES BIOSENSOR**

## **SCOPE OF THE INVENTION**

An aspect of this invention relates to a biosensor for use in determining  
5 analyte presence or concentration in a sample. The biosensor includes a test  
surface which may be used in a variety of assay techniques, such as  
competition assays, sandwich assays, direct and indirect assays, hybridization  
assays, polynucleotide assays, nucleotide sequencing assays and the like. The  
use of uniform size, preferably sub-micron particles of metals, polymers,  
10 glasses, ceramics, phages and the like having dielectric, paramagnetic or  
phosphorescent properties provide a cost competitive, readily manufactured,  
easy to use assay devices. The test surfaces of the devices may be  
manufactured in a manner which only require the addition of a sample for  
purposes of determining suspected analyte in a sample. The test surface is  
15 designed to determine analyte concentration by virtue of addition or  
displacement of the labelling entities to or from the test surface so that a  
change in label signal can be detected. The particle reporters may be  
dielectric particles, paramagnetic particles, phosphorescent particles, phages  
and phages incorporating reporter material. Novel particles for use in  
20 various assays include phage reporter particles and phage reporter particles  
which express on their surface or incorporate solid phase materials or  
molecules which provide dielectric properties, magnetic properties,  
phosphorescent properties and mixtures thereof.

## **BACKGROUND OF THE INVENTION**

25 The greatest challenge in the biosensor industry is to detect in a  
meaningful commercially viable manner assay reactions which take place at  
the molecular level. There are many situations where the detection of analyte  
in sample at concentrations in the picomolar range may be indicative of a  
particular condition in microorganisms and higher life forms, including of  
30 course animals and humans. Such demands have driven the biosensor  
industry towards very sophisticated and elaborate assay schemes to achieve  
such minute detections. However, such devices are normally too expensive  
to manufacture, sell and use and hence are not readily received by the  
research and health care communities.

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Various assays which involve amplification of the signal have been effective in sensing picomolar concentrations. However, such amplification involves fairly elaborate chemistry, several sample handling and washing steps and very sophisticated sensing technology such as fluorometric  
5 detection. An example of such fluorometric amplification is described in U.S. Patent 5,262,299 entitled Enzyme-Amplified Lanthanide Chelate Luminescence.

Other attempts to achieve an amplified signal indicating presence of analyte, involves the use of particles, normally polymeric, gold, solid or  
10 porous glass particles of the micron and sub-micron size. Detection based on the presence of such particles involves the use of filters, capacitance, fluorescence, magnetic separation and the like. In order to achieve the desired sensitivities in using such particles in biosensors it has been a requirement that the operation of the test device be closely controlled. Quite  
15 often the sensitivities in these various types of biosensors are greatly altered by minor external changes such as in temperature, pressure, flow rates, sample introduction and washing times and the like. Even with special care in providing instrument stability it is still not possible to detect changes at the molecular level where just a few molecules are detected.

20 Phosphorescent properties have been used in various types of assays, particularly with the use of metallo-porphyrins. Such compounds are encapsulated in liposomal vesicles or entrapped in polymer latex particles and bound adhesively or covalently to antibodies. The significant disadvantage in the use of porphyrin based phosphors is that the phosphorescence is quenched  
25 by oxygen in aqueous media. An attempt to eliminate this quenching is described in U.S. Patent 5,464,741 where a complex formation with the porphyrin is made.

U.S. Patent 5,043,265 describes the use of inorganic crystalline phosphor particles such as ZnS activated with silver and  $Y_2O_3$  activated with  
30 europium having charged surfaces bound to a macromolecular biological substance. Such binding of the phosphor particle with the charged surface is by adsorption.

U.S. Patent 4,219,335 describes the use of reactance tags in form of small particles which by virtue of capacitance measurement provides a determination of whether or not analyte is present in the sample. The system is particularly adapted to the use of magnetic particles where the presence of the magnetic particles is measured as a change in inductance.

A more sophisticated capacitance type measurement is described in U.S. Patent 4,822,566. Particles however are not used as labelling entities. Instead, the presence or absence of the complexed antibodies is detected by way of a capacitance type measurement to determine analyte concentration.

However, such system is not reliable from a sensitivity standpoint and must be calibrated for each different analyte to be measured. In addition, liquid handling in this system becomes crucial.

The test system and particles used in the biosensor of this invention overcomes a number of the above problems and at the same time provides an assay system which is sensitive at the sub-picomolar or less range.

Experience with current high volume automated instruments in the diagnostic field has demonstrated that 70 to 90% of the robotic cost, complexity, repeat testing, and electromechanical breakdown is related to liquid handling. The system of this invention may contain all reagents within the assay device in which only one liquid handling step is required during introduction of the sample to the device. The device includes a test surface which readily lends itself to simplified robotics.

### **SUMMARY OF THE INVENTION**

In accordance with an aspect of the invention, a displacement competition assay is provided for determining analyte concentration in a sample, the assay comprises:

- i) incubating a sample on a test surface having analyte recognition molecules and labelling entities associated with the recognition molecules, the labelling entities having particle reporters selected from the group consisting of dielectric particles, magnetic particles, phosphorescent particles, particles having a combination of at least two of dielectric, magnetic and phosphorescent properties, phages, phages incorporating reporter material and mixtures thereof, the labelling entities providing a base level signal;

- ii) the sample analyte competing for at least one recognition site on the recognition molecule where binding of the sample analyte to the recognition molecule site displaces the labelling entities away from the test surface to define a test label signal; such displacement of the labelling entities  
5 being related to concentration of analyte in the sample;
- iii) comparing the base label signal to the test label signal to determine concentration of sample analyte.

In accordance with another aspect of the invention, a test component of a diagnostic apparatus for conducting a competition assay comprises a test  
10 surface having recognition molecules for the analyte and labelling entities associated with the recognition molecules, the labelling entities have particle reporters selected from the group consisting of dielectric particles, magnetic particles, phosphorescent particles, particles having a combination of at least two of dielectric, magnetic and phosphorescent properties, phages and phages  
15 incorporating reporter material and mixtures thereof.

In another aspect of the invention, the use of phage particles as reporter labels in identifying the presence of sample analyte in an assay for such detection.

In accordance with another aspect of the invention, the use of semi-  
20 conductor polymer phosphors complexed with labelled recognition molecules as reporter labels in identifying the presence of sample.

In accordance with a further aspect of the invention, a phage reporter particle comprises a phage incorporating reporter material having a property selected from the group consisting of dielectric properties, magnetic  
25 properties, phosphorescent properties and mixtures thereof.

In accordance with another aspect of the invention, an electron beam phosphor excitation apparatus is provided for exciting phosphor labels in a targeted area which optionally lends itself to phosphor label excitation in a grid layout of samples to be individually tested.



**BRIEF DESCRIPTION OF THE DRAWINGS**

Various aspects of the invention are described with respect to the drawings wherein:

Figure 1 is a series of Figures A through C showing the use of particles, particularly phage particles, in a competition assay;

Figure 2 is a series of Figures A through C showing the use of phosphor particles in a sandwich assay;

Figure 3 is a top view of an interdigitated planar capacitance sensor;

Figure 4 is a side view of the sensor of Figure 3;

Figure 5 is a perspective view of two of the capacitance sensors of Figure 3 placed face to face and having a capillary for a volume of the sample space provided therebetween;

Figure 6 is a section through an alternative structure for the capacitance sensor, two interdigitated capacitors being formed back to back upon the same substrate;

Figure 7 shows a side view of a robotic gripper inserted into a semi-micro cuvette, making electronic signal connection to the contact pads of the capacitance sensor of Figure 6;

Figure 8 is a section through an alternative capacitance sensor surface commonly referred to as an open face capacitor for detecting presence of absence of phage reporter particles;

Figure 9 illustrates an example of evanescent wave excitation of solid state phosphor particles;

Figure 10 is a block diagram view of the instrumentation for measuring reactants in capacitance sensor;

Figure 11 is a block diagram of a source compensated mechanical chopper phosphorimeter used in measuring phosphorescence from a solid state or solid phase phosphor particle based labelling entity;

Figure 12 is a plot of sensor capacitance and sensitivity, as a percent change in capacitance, versus thickness of the analyte binding layer on devices of thick film ferroelectric Barium Titanate, or thin film Silicon Nitride dielectric with glass passivation layers;

Figure 13 is a plot of sensor capacitance and sensitivity, as percent change in capacitance, versus thickness of the analyte binding layer on devices having no ferroelectric with glass passivation, or thick or thin films of Barium Titanate ferroelectric without glass passivation;

5        Figure 14 is a plot of capacitance and sensitivity, as percent change in capacitance, for a thin film planar interdigitated capacitance biosensor with various combinations and thicknesses of Barium Titanate, Silicon Nitride, Silicon Oxynitride, and glass;

Figure 15 is a schematic of one element of an array of pulsed  
10    microscale plasma sources generating an electron beam in air which excites a phosphor-labelled recognition molecule bound to an analyte attached to a transparent conductive plate, the phosphorescence being detected through the plate by a photodetector;

Figure 16 is an enlarged view of the transparent conductive plate  
15    illustrating a method of alkoxysilane covalent binding of recognition molecules, exemplified by a nucleic acid sequence, to the surface thereof, and the mechanism of excitation of a semiconductor phosphor label attached to one of the recognition molecules, also exemplified by a nucleic acid sequence hybridized to a complementary sequence bound to the plate;

20        Figure 17 is a schematic of a plurality of electron beam generators focused on a two-dimensional array of test substrates, similar in form to a field emission display device having pixel elements individually addressable by row and column electronic switching;

Figure 18 is a schematic of an electron gun which is capable of  
25    focusing an electron beam on at least two or more positions of a two-dimensional array of test substrates, in form, similar to a cathode ray display in which the electron beam may be addressed to any individual pixel element;

Figure 19 is a graphical representation of the data presented in  
Example 1; and

30        Figure 20 is a graphical representation of the data presented in Example 2.



**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

In accordance with this invention, particle reporters are provided as labelling entities for use in a variety of biosensors. The particle reporters greatly enhance the sensitivity of the biosensors and are capable of detecting the addition or subtraction of a single particle reporter to or from the test surface. This advantage of the invention is due in part to the uniform dimensioned minute particles of micron and nanometre size. The reporter particles may be selected from the group consisting of dielectric particles, magnetic particles, solid-phase phosphor particles, particles having a combination of at least two of dielectric, magnetic and phosphorescent properties, phages, phage incorporating reporter material and mixtures thereof. The reporter particles can be used in a variety of assays which include competition, sandwich, direct, indirect immunoassays and nucleic acid hybridization assays and nuclear acid sequencing assays. Exemplary immunoassays which involve the use of analyte recognition molecules for recognizing analyte in the sample are shown in Figures 1 and 2.

In Figure 1A a competition assay is shown where a test surface 10 has analyte entities 12 linked to the test surface 10 by linking components 14. The analyte recognition molecule 16 has a reporter particle 18 which as the labelling entity is attached to the recognition molecule 16. The analyte entity 12 may be the same as the analyte of the sample to be detected or may be a fragment or some other analog thereof. The analyte entity 12 may have the same binding affinity for the analyte recognition molecule 16 or may have a greater or lesser binding affinity than the analyte in the sample for the recognition molecule 16.

In Figure 1B the sample 20 is introduced and contains analyte 22. Analyte 22 competes with analyte entity 12 for the analyte recognition molecule 16. The concentration of the analyte 22 in the sample 20 determines the extent to which the analyte molecules 22 displace the analyte entities 12 from the recognition molecules 16 so as to compete with the analyte entities in binding to the recognition molecules 16. As shown in Figure 1C the analyte in the sample has competed with the analyte entities 12 to displace the labelling entity 18 away from the test surface 10. Such

displacement of the labelling entity from the test surface results in a measurable difference, hence determining through suitable controls of the and biosensors, both a qualitative and quantitative measure of the analyte present in the sample.

- 5       As shown in Figure 2, a biosensor 24 has a capillary space 26 defined between biosensor plate 28 and test surface 30. The test surface is a layer of recognition molecules 32 linked to the surface 30 by linkers 34. On the undersurface 36 of the plate 28 is a dried film of labelling entities 38 which comprise antibodies 40 having linked thereto phosphor reporter particles 42.
- 10   When the sample is introduced to the capillary space 26, which may be a drop of whole blood 43, the blood 43 is drawn into the capillary space 26 by capillary attraction. The presence of the blood dissolves the labelling entities 38 from the underside 36 of plate 28. The analyte 44 in the sample has a site 46 which is recognized by the analyte recognition molecules 32 and a site 48
- 15   which is recognized by the antibodies 40. The analyte 44 is then sandwiched between the recognition molecules 32 and the fixed antibodies 38. As the sample is incubated in the biosensor 24, analyte molecules 44 are bound to the recognition molecules 32 and are correspondingly labelled with the particle reporters 40. By virtue of the antibodies recognizing the sites 48 on
- 20   the analyte, the phosphor particle reporters are immobilized on the test surface 30. Such sandwich assay then provides in an appropriate biosensor, a measure of analyte present on the test surface 30.

The use of uniform size nanoparticles or micron particles as particle reporters can provide in suitably calibrated biosensor, a measure in terms of

25   presence or absence of the reporter particles down to a level of one reporter particle. By selecting the properties of the reporter particle, its dielectric paramagnetic or phosphorescent properties can be enhanced relative to the sensing conditions of the biosensor. It is now possible to obtain in nanoparticle size a variety of particles made from ceramics, metal oxides,

30   plastics, glasses and the like. Kossovsky et al. U.S. Patent 5,219,577 describes ruthenium oxide, tin oxide and glass nanoparticles which may be coated with cellobiose to form a strong adhesive bond with glass ceramic nanostructures. These structures which can be derivatized, may be reacted

with proteins, lipoproteins, glycoproteins, drugs, heptens, oligonucleotides and the like. With nanoparticles the activity of the various biological molecules attached thereto is normally retained. Another suitable linking technique is described in U.S. Patent 5,429,824 where tyloxapole is used as a nanoparticle stabilizer and dispersant. The core particles may be made from a wide variety of inorganic materials including metals or ceramics. Preferred metals and semimetals include Chromium, Rubidium, Iron, Zinc, Selenium, Nickel, Gold, Silver, Platinum, Palladium, Silicon, Germanium, and the like. Preferred inorganic crystalline or ceramic materials include II-VI compound, and group IV semiconductors, Lead Zirconate Titanate, Barium Titanate, Silicon Dioxide, Titanium Dioxide, Aluminum Oxide, Ruthenium Oxide, Zinc Oxide, Cadmium Sulfide, Cadmium Selenide, and Tin Oxide. The core particles may be made from organic materials including the carbon structures, diamond, graphite, and nanospherical and nanotubular fullerenes (such as C<sub>60</sub>, and C<sub>70</sub>). Preferred polymers include semiconducting polymers such as poly(phenylenevinylene), poly(styrene), poly(methylmethacrylate), poly(dimethylsiloxane), polyimides, poly(vinyl alcohol), poly(vinylpyrrolidone), nylon, and polysaccharides such as cyclodextrins, chitosan, dextran, agarose, sepharose and nitrocellulose.

Particles made from the above materials having diameters less than 1000 nanometres are available commercially or they may be produced from progressive nucleation in solution (colloid reaction), or various physical and chemical vapour deposition processes, such as vacuum sputtering deposition (Hayashi, C., J. Vac. Sci. Technol. A5(4), July/Aug. 1987, pgs. 1375-1384; Hayashi, C., Physics Today, Dec. 1987, pgs. 44-60, MRS Bulletin, Jan. 1990, pgs. 16-47). Tin oxide having a dispersed (in H<sub>2</sub>O) aggregate particle size of about 140 nanometres is available commercially from Vacuum Metallurgical Co. (Japan). Other commercially available particles having the desired composition and size range are available from Advanced Refractory Technologies, Inc. (Buffalo, N.Y.).

Plasma-assisted chemical vapour deposition (PACVD) is one of a number of techniques that may be used to prepare suitable microparticles. PACVD functions in relatively high atmospheric pressures (on the order of

one torr and greater) and is useful in generating particles having diameters of up to 1000 nanometres. For example, aluminum nitride particles having diameters of less than 1000 nanometre can be synthesized by PACVD using  $\text{Al}(\text{CH}_3)_3$  and  $\text{NH}_3$  as reactants. ( The PACVD system typically includes a horizontally mounted quartz tube with associated pumping and gas feed systems. A reactor is located at the centre of the quartz tube and heated using a 60 KHz radio frequency source. The synthesized aluminum nitride particles are collected on the walls of the quartz tube. Nitrogen gas is used as the carrier of the  $\text{Al}(\text{CH}_3)_3$ . The ratio of  $\text{Al}(\text{CH}_3)_3:\text{NH}_3$  in the reaction chamber is controlled by varying the flow rates of the  $\text{N}_2/\text{Al}(\text{CH}_3)_3$  and  $\text{NH}_3$  gas into the chamber. A constant pressure in the reaction chamber of 10 torr is generally maintained to provide deposition and formation of the ultrafine nanocrystalline aluminum nitride particles. PACVD may be used to prepare a variety of other suitable nanocrystalline particles.

Paramagnetic materials are described in nanoparticle form in U.S. Patents 5,468,427 and 5,523,065. The ceramic nanoparticles are substantially homogeneous in composition and in diameter and may be of the order of 300 nm in size. A larger number of metal semiconductor and lanthanide metal oxide nanoparticles may be produced by the method of this U.S. Patent including  $\text{SiO}_2$  nanoparticles.

The core particles are optionally coated with a substance that provides a threshold surface energy to the particle sufficient to cause binding to occur without that binding being so tight as to denature biologically relevant sites. Coating is preferably accomplished by suspending the particles in a solution containing the dispersed surface modifying agent. It is necessary that the coating make the surface of the particle more amenable to protein, peptide, or nucleic acid sequence attachment. Suitable coating substances in accordance with the present invention include polycarboxylic acids, such as EDTA and triethylenetetraminehexaacetic acid, cyclodextrins, cellobiose, related basic sugars, and modified sugars such as nitrocellulose. Oligonucleotides may also be used. Suitable oligonucleotides include polyadenosine (poly A). Cellobiose is a preferred coating material.



The coating solution into which the core particles are suspended contains, for example, from 1 to 30 weight/volume percent of the coating material. The solute is preferably double distilled water (ddH<sub>2</sub>O). The amount of core particles suspended within the coating solution will vary  
5 depending upon the type of particle and its size. Typically, suspensions containing from 0.1 to 10 weight/volume percent are suitable. Suspensions of approximately 1 weight/volume percent of particles are preferred.

The core particles are maintained in dispersion in the coating solution for a sufficient time to provide uniform coating of the particles. Sonication is  
10 the preferred method for maintaining the dispersion. Dispersion times ranging from 30 minutes to a few hours at room temperature are usually sufficient to provide a suitable coating to the particles. The thickness of the coating is preferably less than 5 nanometres. Thicknesses of the coating may vary provided that the final core particles include a uniform coating over  
15 substantially all of the particle surface.

The particles are separated from the suspension after coating and may be stored for future use or redispersed in a solution containing the protein or peptide to be attached to the particles. Alternatively, the coated particles may be left in the suspension for further treatment involving attachment of the  
20 desired protein or peptide.

In particular, surface active agents for modifying nanoparticle surface properties through adsorptive coating involve the use of cellulose derivatives such as methyl, carboxymethyl, hydroxyethyl, hydroxypropyl and hydroxypropylmethyl as used in the preparation of derivatized nanoparticles.

25 Covalent linkers for nanoparticles involve covalent immobilization of protein, glycosidic and lipidic structures to the solid phases. Such techniques preserve the tertiary structure and biochemical function of the analyte entity and/or recognition molecules. Preferably, they orient the recognition molecules in such a way as to favour their complex formation, such as  
30 described in the linking entity used in Newman U.S. Patent 4,822,566.

Commonly, -OH functional groups are introduced to the surfaces of glasses, (eg. SiO<sub>2</sub>), semiconductors, metal oxides, metals and polymers by treatment of the surfaces under acid conditions as described in U.S. patent

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4,824,529. These -OH groups readily react with trifunctional silanes such as (3-aminopropyl) triethoxysilane or 3-glycidoxypropyltrimethoxysilane, or with monofunctional aminosilanes such as (4-aminobutyl)dimethylmethoxysilane, or with thiol-terminal silanes, such as mercaptomethyltrimethoxysilane, or trihexylchlorosilane, the latter by nucleophilic displacement of the chlorine. The linkers are available commercially from such suppliers as Fluka (Hauptmann, NY), Aldrich (Milwaukee, WI), and Petrach Systems (Bristol, PA). To these amino- or thiol-terminal silanes one may graft the desired peptide, lipidic, or glycosidic moiety via homobifunctional crosslinkers such as glutaraldehyde, or via heterobifunctional crosslinkers such as N-γ-maleimidobutyryloxy succinimide ester (GMBS) (Calbiochem, San Diego, CA.), or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Pierce, Rockford, IL.). Synthetic peptides, and libraries may be grown on these silane-derivatized solid phase particles and surfaces by any of the peptide synthesis chemistries (eg. t-BOC, Fmoc chemistries) which are based mostly on the reaction of active ester intermediates of N-protected amino acids or amino acid analogs in solution phase with deprotected amino acids linked to a solid phase (SiO<sub>2</sub>, sepharose, etc.)

The particles can be bonded to recognition molecules, analyte or analyte entities by known techniques. The particle reporters can be encapsulated with an organic polymer to which the immune compounds can be bonded, or the particle surface can be silanized by conventional techniques as described above. Organic compounds can then be attached to the silane linkage. U.S. Patent 3,954,666 teaches polymer encapsulation of core materials and U.S. Patent 3,983,299 teaches the use of silane linkages to bond organic compounds to inorganic particles. Techniques for immobilization of enzymes on magnetic supports which would be equally applicable to antibodies are described in Enzymology, XLIV pp. 324-326. Other bonding methods may also be used so long as they do not interfere with the complexing ability of the recognition molecules. The recognition molecules may be chosen to be specifically reactive with either the analyte or with the complex of the analyte with another recognition molecule.



Recognition molecules include not only antibodies, but also receptor proteins, receptor ligands, serum proteins, DNA binding proteins, DNA, RNA, lectins or virtually any molecule that binds specifically to a complementary moiety. The recognition molecule may be coupled to the particle by a variety of means, the optimal conditions depending on the combination of the recognition molecule and the particle. In accordance with one aspect of the invention, antibody molecules are adsorbed to a phosphor nanoparticle. It has previously been shown by Tanke (U.S. Patent 5,043,265) that adsorption of antibodies to phosphors is preferred to covalent coupling via Silane spacer molecules. However, a major problem with this technique is instability. Molecules prepared in this manner are not sufficiently robust to withstand the rigorous pipetting and washing steps associated with a typical immunoassay. The current invention describes the crosslinking of the surface adsorbed antibodies via homobifunctional crosslinkers such as glutaraldehyde. This results in a "cage" of antibodies or other recognition molecules surrounding the phosphor particle. The crosslinking of the antibody around the phosphor does not affect the binding of the "caged" phosphor versus an "uncaged" phosphor in terms of specific reactivity nor does it have a quenching effect on the phosphorescence of the particle. Also, having a "cage" around the particle obviates the need to apply charges to the surface of the phosphor particles as taught by Tanke (U.S. Patent 5,043,265) to avoid the formation of large aggregates. Tanke teaches that the phosphors should be subjected to a pretreatment to provide their surface with charged groups such that as a consequence, the individual particles will repel each other and the formation of large aggregates will be avoided. Treatment with the crosslinker is performed under standard conditions to avoid aggregation (e.g., agitation, etc.). In addition, it provides a major advantage in terms of stability and product consistency.

The receptor molecule can be attached to the test surface by surface adsorption, gel entrapment, covalent bonding or other similar methods. Of these, covalent bonding is preferred. Any system of recognition molecule attachment capable of orienting the molecules on the test surface so that they will have maximum activity is also preferred. Recognition molecules include

not only antibodies, but also tissue or cellular receptor proteins, serum transport proteins, binding proteins, peptides, DNA, RNA and lectins. Other classes of compounds, such as chelating agents, may also serve as useful receptor reagents if they react with the substance to be determined in the body fluid with sufficient specificity to avoid false results caused by competing reactions.

The binding agent that forms the biochemical binding system can be selected from general or specific affinity ligands and may include, but is not limited to, antibodies, antibody fragments, binding proteins, lectins, enzymes and receptors. The immobilized analyte which forms the first layer of the biochemical binding system may be the same molecular substance as the analyte under test, or it may be an analog of the analyte that is biospecific to the binding agent. The immobilized analyte may, for example, be an antigen, a hapten, a polysaccharide, a glycoprotein, a glycolipid, an enzyme inhibitor, an enzyme substrate, a neurotransmitter, a hormone, a polynucleotide and the like. Table I contains examples of the biochemical binding systems which may be used in a competitive binding embodiment to test for a particular analyte.

**Table I**

Immobilized analyte	Binding Agent	Analyte	Class of Sensor
antigen	antibody	antigen	A
haptten	antibody	haptten	A
polysaccharides	lectin	polysaccharides	B
glycoproteins	lectin	glycoproteins	B
glycolipids	lectin	glycolipids	B
enzyme inhibitor	enzyme	enzyme inhibitor	C
enzyme substrate	enzyme	enzyme substrate	C
enzyme inhibitor	enzyme	enzyme substrate	C
neurotransmitters	neural	neurotransmitters	D
	receptor		
hormones	tissue/neural	hormones	D
	receptor		
proteins	single & double stand DNA & RNA	protein DNA, RNA	E
vitamins	binding proteins or DNA	vitamins	E
DNA	DNA/Antibody	DNA/RNA	E
RNA	RNA/Antibody	DNA/RNA	

As can be seen from Table I, there are four classes of the competitive binding sensors. In class A the binding agent is an antibody specific to the analyte. The analyte may be an antigen or hapten. The biochemical binding system comprises a first immobilized layer of the antigen or hapten analyte  
5 with a second layer of the biospecific antibody biochemically bound to the immobilized analyte in the first layer.

In class B, the binding agent is a lectin, which is a general ligand specific to a group of analytes. A lectin-based sensor can be made more specific by an appropriate molecular sieve membrane that excludes larger  
10 molecules in the general analyte group from reacting with the biochemical binding system. In this class, for example, the binding system could have a first immobilized layer of polysaccharide or a membrane protein containing sugar residues of certain configurations and a second layer of the general lectin bound to the first layer.

15 In class C, the binding agent is an enzyme reactive with an enzyme inhibitor or enzyme substrate. In this class, for example, the binding system could have an inhibitor for a particular enzyme immobilized on the sensor surface and a second layer containing the enzyme bound to the inhibitor in the first layer. With a particular enzyme substrate in the test fluid, the enzyme  
20 binding agent will be drawn from the surface of the binding system.

In class D, the binding agents are neuroreceptors and tissue receptors. The receptor has its molecular conformation greatly altered by various neurotoxins and other agents. The binding system can have a layer of succinylcholine immobilized on the sensor surface with a second layer of  
25 acetylcholine receptor molecules bound to the first layer. If neurotoxins, for example, is present in the test fluid, the receptor binding behaviour will be altered and it will be released from the binding system surface, thereby altering the dielectric properties of the sensor. It is of course to be understood that these are merely examples of the biochemical binding systems  
30 that can be used with the competitive binding embodiment of the present invention.

In class E, DNA/RNA are the binding agents to which targeted DNA/RNA hybridizes as, for example used in nucleotide diagnosis or in nucleotide sequencing. It is also understood that it is now possible to raise certain types of antibodies to nucleic acid sequences and that there are  
5 specific DNA and RNA binding proteins, such as "zinc-finger" proteins. Hence, the binding agent may also be an antibody. The target DNA/RNA (analyte) may encode known proteins, vitamins and fragments thereof.

Molecules of a binding agent 16 are immobilized on the passivation layer on test surface with linking molecules 14. In Fig. 1A, the layer of the  
10 immobilized binding agent coats the entire passivation surface 10. The binding agent is an affinity ligand that will bind specifically to the analyte, such as an antibody binds specifically to a particular virus or as an antigen binds specifically to a particular antibody. Alternatively, the affinity ligand may bind to a specific group of analytes, such as nucleotide analogs and  
15 lectins bind to certain groups of biochemical analytes.

The materials useful as capacitance particle reporters which combine with recognition molecules or analyte entities are those which will alter the electrical reactance of the test surface. That is, these materials, if distributed as a finely divided powder on the test surface, alter the dielectric, conductive  
20 or the magnetic properties of the surface. The advantage of this invention resides in using particles which are detectable in very small quantities by very sensitive but well developed and readily available electrical components. Further, the use of such materials avoids the problems of transient activity and handling hazards which one encounters in the use of radioactive tags.  
25 The preferred materials for detection by inductive reactance are metals and metal oxides which exhibit paramagnetism. Such materials can be bound to antibodies and applied to the test surface while in a demagnetized state. Once they have been applied, the entire surface can be exposed to a magnetic field to magnetize the particles for detection purposes. This magnetic activity is  
30 then readily detectable by various well-known means such as a standard magnetic tape read head or Hall effect detector. Magnetic materials include, but are not limited to, metals and alloys such as iron powder, nickel, cobalt,  $\text{CrO}_2$ , "Ferrofluid" (a ferromagnetic liquid produced by Ferrofluids Corp.),

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$\text{Co}_3\text{O}_4$ ,  $\text{NiO}$ ,  $\text{Mn}_2\text{O}_3$ , magnetoplumbites, magnetic iron oxides and "Alnico", an alloy of aluminum, nickel, cobalt and copper. Preferred magnetic materials are the magnetic oxides of iron, cobalt, nickel, chromium and manganese and oxide coated particles of iron or nickel.

- 5        Other useful materials are organic charge complexes with high electrical conductivity such as N-methylphenazinium, tetacyanoquinodimethane,  $[\text{Ce}(\text{NO}_3)_6\text{Mg}(\text{H}_2\text{O})_6]_3 \cdot 6\text{H}_2\text{O}$  crystal,  $\text{Bi}_2\text{Se}_3$  crystals and tetrathiafulvalene complexes with tetracyano-p-quinodimethane or  $\text{K}_2\text{Pt}(\text{CN}_4)\text{BrO}_3 \cdot \text{H}_2\text{O}$  and amorphous materials with magnetic properties such
- 10 as the chalcogenides, e.g., the europium chalcogenides and chalcogenide glass particles.

The solid phase phosphor particles may be made in the micro or nanoparticle size. Suitable solid phase phosphors may include nanoparticulate amorphous silicon, or compounds of the following

15 formulations:

- Inorganic crystalline II-VI compound semiconductor host crystals of the formula  $\text{MA}$  where  $\text{M}$  is  $\text{Zn}$ , or  $\text{Cd}$ , and  $\text{A}$  is  $\text{O}$ ,  $\text{S}$ , or  $\text{Se}$ , this host crystal being doped with typically less than 5% of  $\text{Mn}$  or a lanthanide including  $\text{Eu}$ ,  $\text{Sm}$ ,  $\text{Tb}$ , or  $\text{Dy}$
- 20 • Phosphors which can be represented by the formula  $\text{MA}_2\text{O}_4$  in which  $\text{M}$  is a metal from the group  $\text{Ca}$ ,  $\text{Sr}$  and  $\text{Ba}$ , or in which  $\text{M}$  is  $\text{Mg}$  in combination with one or more of  $\text{Ca}$ ,  $\text{Sr}$ , or  $\text{Ba}$  with  $\text{Eu}$  as "activator".
- 25 • Phosphors which can be represented by the formula  $\text{M}_2\text{AOF}_5$  where  $\text{M}$  is  $\text{K}$ ,  $\text{Ru}$  or  $\text{Cs}$ , and  $\text{A}$  is  $\text{Ta}$ , or  $\text{Nb}$ .
- 30 • Polymorphous phosphorescent compounds which can be represented by the formula  $\text{MNbOF}_5$  where  $\text{M}$  is one or more of  $\text{Ba}$ ,  $\text{Sr}$ , and  $\text{Pb}$ .



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- Polymorphous phosphorescent compounds which can be represented by the formula  $MWO_2$  where M is one or more of Ba, Sr, or Pb.
- 5     • Phosphorescent compounds which can be represented by the formula  $Pr_xSrM_{1-x}AlO_4$  where Pr is Praseodymium, M is La (Lanthanum III) or Ga (Gadolinium III), and X is in the range 0.025 to 0.075.
- 10    • Phosphorescent compounds which can be represented by the formula  $NaY_{1-x}A_xM_2O_6$  where A is Eu, or Bi (bismuth), M is Zr (Zirconium) or Hf (Hafnium), and X is in the range of 0.001 to 0.25.
- 15    • Phosphors based upon a crystalline matrix of an alkaline earth and a chalcogen together with an activator prepared from both Se and S vapours.
- 20    • An infrared light excitable phosphor prepared from a base, first and second dopants, a carbonate of the base, and a fusible salt. The base is an alkaline earth metal sulphide such as CaS or SrS.  $CaCO_3$  or  $SrCO_3$  is the corresponding carbonate of the said base and LiF is used to allow the material to be fused together. Samarium oxide, and Europium oxide are used as the first and  
25    second dopants.
- 30    • An infrared light up-converting phosphor, which emits visible light of a wavelength shorter than the exciting wavelength, wherein the host crystal is of the formula  $M_2O_2S$ , M being one of Y, Gd, Lu or La, and wherein the host is doped with activator couples consisting of the infrared absorber Ytterbium together with an emitter including, but not limited to, Er, Tm, Ho, or Tb.

A further benefit in using phosphor particles is that their composition may be selected by the use of various dopants in a way to determine the colour of the phosphorescence. Preferred semiconductor polymers include polyvinylcarbazole (PV/C) and phenylmethyldopolysilane (PMPS). Preferred  
5 inorganic phosphorescent host crystals include ZnO, ZnS, CdS, Y<sub>2</sub>O<sub>3</sub>, Y<sub>2</sub>O<sub>2</sub>S, and SrS. Preferred dopants of these host crystals include Manganese, Cerium, Terbium, Europium, Samarium, Thulium, and other Lanthanides such as Dysprosium. Four different colour emissions which could be used in DNA sequencing to identify the four individual nucleotide bases. This permits each  
10 nucleotide substrate to be "labeled" with a phosphor emitting in a separate region of the UV-Visible-IR spectrum. Each would be measurable with high discrimination, and at very low concentrations by optoelectronic means.

The phosphor particle for use in this invention may be an electro/photo-luminescent band-gap (semiconductor) polymer, or a solid state  
15 inorganic phosphor, including the atomic series group IV and II-VI compound semiconductors. The test surface containing the phosphor particle reporters may be excited to phosphoresce by use of suitable electromagnetic radiation. The test surface may be part of a wave guide which carries the electromagnetic radiation such that any phosphor particles adjacent the test  
20 surface will be excited by the radiation travelling along the wave guide to emit detectable phosphorescence. Examples of phosphor materials are described in U.S. Patents 4,830,875; 4,879,186; 5,122,305 and 5,227,254.

The dopant impurity of the band-gap polymer may be an atom, another smaller organic or inorganic solid state nanoparticle phosphor, a lanthanide  
25 chelate, a lanthanide cryptate or other similarly "caged" lanthanide ion or atom.

Such an organic phosphor comprises of molecules of at least one phosphorescent activator within a condensation polymer that does not have an absorption spectrum overlapping that of the phosphor's excitation or  
30 emission bands. The phosphor activator is a compound or mixtures of compounds of the general formula (A)<sub>m</sub>--Ar--CO--X, where Ar denotes a

fused ring polycyclic aromatic group; m is zero or an integer of from 1 up to the maximum number of ring positions available for substitution; X denotes an alkyl group or a group of the formula  $(A)_m\text{--Ar--}$  or  $(B)_n\text{Ph--}$ , where Ph is a phenyl group and n is zero or an integer of from 1 to 5; and A and B represent ring substituents such as methyl, amino, carboxyl, and mercapto groups.

Inherently photoluminescent and electroluminescent polymers include poly(paraphenylene), parahexaphenyl, poly(peryene-co-diethynylbenzene) [Tasch S. et. al. The Applications of Poly(phenylene) Type Polymers and Oligomers in Electroluminescent Color Displays. Proceedings of the Materials Research Society. Vol. 471: 325 - 330, 1997]. Others in this class are poly(aniline) (Emeraldine), poly(9-vinylcabazole), poly(phenylenevinylene), poly(thienylene), poly(thienylenevinylene), poly(phenylene sulfide), poly(9,9-dihexylfluorene), and poly(methylphenylsilane) [Suzuki H. et. al. Charge carrier and exciton dynamics in polysilane-based multilayer light-emitting diodes as monitored with electroluminescence. J. Luminescence 66 and 67: 423 - 428, 1996.]

Insulating, conducting or semiconducting polymers may be doped with charge generating compounds such as 1) Phthalocyanines and Naphthalocyanines, 2) metalloporphyrins, 3) Thiapyrylium, 4) Perylenes, 5) Quinacridones, 6) Squarilium (e.g. 1,3-bis[4-(dimethylamino)phenyl]-2,4-dihydroxycyclobutenediylum), 7) 9,10-bis(styryl)anthracene [Saito S. et. al. Progress in organic electroluminescent materials and device structures. J. Luminescence 60 and 61: 902 - 905, 1994.]

Electron and hole transporting organic polymers, or semiconductor polymers are appropriate hosts for phosphorescent dopants. In general cyclic C, N, O structures are electron transporters. These include poly(oxadiazols) such as: 1) 2-(4-biphenyl)-5-(4-tert-butylphenyl)-1,3,4-oxadiazole, and 2) 1,3,5-tri-(4-tert-butylphenyl-1,3,4-oxadiazolyl)benzene [Shirota Y. et. al. Starburst molecules based on  $\pi$ -electron systems as materials for organic electroluminescent devices. J. Luminescence 72 -74: 985 - 991, 1997]. Also included in this class are 1) fluorenylidene malononitrile, 2) diphenoquinones, 3) fullerenes, and 4) Aluminum quinolates

Hole transporters include poly (triarylamines) such as: 1) tri-*p*-tolylamine, 2) N,N'-bis(3-methylphenyl)-N,N'-diphenylbenzidine, 3) 4,4',4''-tris(diphenylamino)triphenylamine, 4) 1,3,5-tris(diphenylamino)benzene, 5) 4,4',4''-tris(N-carbazolyl)triphenylamine

- 5 Doping of these may be accomplished with phosphorescent or fluorescent polycyclic organic compounds, Lanthanide chelates, or inorganic nanocrystalline semiconductors. Examples of polycyclic organic fluorescent dopants include: 1) N,N'-bis((2,5-tert-butylphenyl)-3,4,9,10-perylenedicarboximide, 2) N,N'-bis((2,6-dimethylphenyl)-3,4,9,10-perylenetetracarboxylic diimide, 3) N,N'-bis((3-aminophenyl)-3,4,9,10-perylenetetracarboxylic diimide, 4) fluorenones (e.g. 2,7-bis[2-(diethylamino)ethoxy]-9-fluorenone), 5) dibenzothiophenes (e.g. 2,8-bis(dimethylaminoacetyl)-dibenzothiophene)
- 10

- Examples of inorganic nanocrystalline doping of polymers include: 1) ZnS:Mn, CdS:Mn, or ZnS:Tb nanoclusters formed in the voids of the diblock copolymer of methyltetra cyclododecane, and 2-norbornene-5,6-dicarboxylic acid. [ Kane R.S, et. al. Photoluminescent Mn-Doped ZnS Nanoclusters Synthesized Within Block Copolymer Reactors. Proceedings of the Materials Research Society. Vol. 471: 313 - 317, 1997]. 2) Copolymerization of Zinc methacrylate with styrene initiated with azoisobutyronitrile to form a gel, and subsequently forming entrapped ZnS nanocrystals by treatment of the gel with H<sub>2</sub>S. [ Yang Y. et. al. Preparation, Characterization and Electroluminescence of ZnS Nanocrystals in a Polymer Matrix. J. Materials Chemistry 7 (1): 131 - 133, 1997].
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- 20

- 25 Examples of Lanthanide (Europium, Terbium, Samarium, Dysprosium) chelates include the  $\beta$ -diketones which are often combined together with 1,10 phenanthroline, 1,10 phenanthroline -N-oxide, or 2,2'-bipyridyl. Representative  $\beta$ -diketones include 1) 1-phenyl-1,3-butanedione, 2) 4,4,4-trifluoro-1-phenyl-1,3-butanedione, 3) 1,1',6,6'-tetramethyl-3,5-hexanedione [Erotyak J. et. al. Time-resolved study of intramolecular energy transfer in Eu<sup>3+</sup> Tb<sup>3+</sup> / $\beta$ -diketone/o-phenanthroline complexes in aqueous micellar solutions. J. Luminescence 72 -72: 570 - 571, 1997.] ,
- 30
- [Alves S. et. al. Luminescence and quantum yields of Eu<sup>3+</sup> mixed complexes

with 1-phenyl-1,3-butanedione and phenanthroline or 1,10 phenanthroline-N-oxide. J. Luminescence 72 -74: 487 - 480, 1997.], [ Batista H. et. al. Synthesis, crystal structure determination and theoretical prediction of the structure and electronic spectrum of Eu(btfa)<sub>3</sub>bipy. J. Luminescence 72 -74: 5 159 - 161, 1997.], [Malta O. et. al. Intensity parameters of 4f - 4f transitions in the Eu(dipivaloylmethanate)<sub>3</sub> 1,10 phenanthroline complex. J. Luminescence 69: 77 - 84, 1996.], 4) 2,4-pentanedione, 5) thenoyltrifluoroacetone, and 6) 1,3-diphenyl-1,3-propanedione. Europium and Terbium fluorescence is also increased in the polyester matrix polyethylene phthalate by addition of the co-  
10 dopant pyrazine-2-carboxylic acid [dos Ajos P. et. al. Spectroscopic properties of trivalent europium and terbium ions in modified polyethylene phthalate with aza-aromatic acids. J. Luminescence 72 - 74: 487 - 489, 1997]. Other chelators of Lanthanides include 1) 8-hydroxyquinoline chelates, and 2) fluorosalicylic acid - EDTA [Christopoulos T., and Diamandis E.  
15 Enzymatically amplified time-resolved fluorescence immunoassay with terbium chelates. Analytical Chemistry 64: 342 - 346, 1992.]

In a preferred embodiment, lanthanide ion co-ordination labels are applied. The Trisbipyridine Cryptate "cage" for Eu<sup>3+</sup> structure synthesized by J-M Lehn provides a coordination sphere of 8 Nitrogen atoms and two  
20 pendant (primary amino and hydrazide) covalent conjugation groups. Cryptate-based fluorescence resonance energy transfer (FRET) assays for clinical analytes were introduced with the CIS Biointernational "Kryptor" instrument. Detection of PCR-amplified papillomavirus DNA sequences in clinical smears by cryptate-labeled nucleotides has also been described.

25 The use of further improved polyaminocarboxylate chelators as reported by Mondry et.al. [(1994) J. Luminescence 62:17] who demonstrated complete exclusion of solvent from the coordination spheres of both Eu<sup>3+</sup> and Tb<sup>3+</sup> by Triethylenetetraminehexaacetic Acid (TTHA) is included. Kim et al. [(1994) J. Luminescence 62:173] also showed that -COOH side groups  
30 of poly(styrenemaleic acid) (PSMA) copolymer effectively isolated the Lanthanides Tb, Eu, Sm, Gd, Yb and Dy from water by directly coordinating to the Ln<sup>3+</sup> ions. In this doped polymer the styrene moiety provided the UV sensitizer, enhancing the Lanthanide ion phosphorescence between 100 and



750 times. The photoluminescence of the PSMA itself was enhanced and blue-shifted upon coordination to all but  $\text{Eu}^{3+}$  and  $\text{Yb}^{3+}$ .

Solvent O-H, C-H, and N-H non-radiative quenching of the lanthanide ion triplet state is recognized to be the critical problem in obtaining high  
5 quantum efficiency.

In one aspect of the invention, we have applied recently-developed nanostructured bandgap materials as labels in biological/biochemical assays.

There is a growing class of band-gap polymers which may be suitable as "caging" and sensitizing structures for lanthanide ion dopants. The  
10 example of polystyrenemaleic acid is cited above. Photoluminescent [Delgado, G.R. et al. (1997) Proc. Material Research Society, Abs. p.137] and charge transporting [Wang Y. and Herron N. (1996) J. Luminescence 70:48] polymers having a band-gap of 3 to 5 electron volts (400 to 250 nm) can also act as exciton or electron donors for embedded group IV and II - VI  
15 compound semiconductor nanocrystal phosphors. PVK (polyvinylcarbazole), and PMPS (phenylmethylpolysilane) are representative of this class of semiconducting polymers. Furthermore it is straightforward to derivatize and couple them to proteins and nucleotides. While the experimental evidence of this application is on the inorganic host crystal, band-gap polymers and their  
20 potential for either direct lanthanide doping or nanocrystal phosphor encapsulation are included.

Ideally one wants a nanoparticle phosphor which:

- behaves as a general purpose reagent in biochemical buffers and media, providing both adhesive and covalent  
25 means of conjugation;
- gives a quantum yield comparable to or better than conventional fluorophors, organic chelates/cryptates of lanthanide ions, platinum/palladium-porphyrin derivatives;
- in a single type of host crystal (a single optimal excitation  
30 wavelength) allows doping with 4 or more lanthanide ions such that 4 or more distinct non-overlapping fine line emission spectra can be obtained;



- can be size selected to have a narrow size dispersion, permitting uniformity of migration in chromatographic processes, electrophoresis, and gradient separation, so that attached molecules, rather than the particles themselves, determine separation behavior.

Control of surface chemistry generally involves molecular "capping" of the nanocrystal phosphor in such a way that particles do not form superlattice aggregates (micron-size or larger).

One of the most studied aspects of undoped nanocrystalline phosphors is the "blue shifting" of emission peak and absorption spectrum band edge. Peak wavelength decrease in proportion to the decreasing crystal diameter has been observed in both group IV semiconductors (Si, Ge), and compound semiconductors CdSe, CdS. Delgado et. al. have reported that Ge nanoparticles embedded in PVK, PMMA or silica sol-gels provide size-tunable emission from 650 nm (deep red) for 90Å to 340 nm (UV) for 10Å particles. Whether particles are intended for laser media or assay labels, having narrow size dispersion in undoped crystals is important in reducing the corresponding dispersion of the emission peak wavelength and bandwidth. Being able to control the size distribution of the particle means being able to precisely select (tune) the color and maximize the gain of a nanocrystalline laser. In immunoassay and nucleic acid hybridization assays it means being able to select multiple analyte-specific phosphor labels which have non-overlapping emission spectra. The luminescence or autofluorescence of these host band gap materials has been attributed to deep trap states and crystal defects as opposed to surface states or oxidation. Thus, coupling through surface oxides (functional alkoxysilanes), which will minimally influence the phosphorescence behavior is included in one aspect.

Undoped group IV, and II-VI nanocrystalline semiconductor phosphors (e.g. CdS) retain the long phosphorescence lifetimes of the bulk semiconductor. They also retain the broad emission spectrum of the bulk although the emission peak is blue shifted. Presumably, in the absence of defects or impurities in the quantum-confined lattice, the transition from a conduction band state back to a valence band state is partially forbidden.

Doped semiconductors (e.g. ZnS:Tb less than 5 nm), exhibit extremely rapid trapping from the host conduction band to the impurity's (dopant atom's) excited state. In the case of ZnS:Eu, the phosphorescence half-life decreases by 6 orders of magnitude from milliseconds for the bulk material to  
5 nanoseconds for the nanocrystalline material.

Capping also ensures that photo/electro-luminescence quantum efficiency is preserved in the biochemical matrix of the assay (i.e., variable quenching of phosphorescence should not occur due to serum pH, ionic strength, and matrix components such as DNA, protein, lipids, drugs, etc.).  
10 ZnS:Mn nanocrystals of about 3.5 nm when treated with surfactant (poly)methylmethacrylate, for example, exhibit emission intensity increases of 4 times or more over the untreated crystal.

The capping molecule will partially or entirely determine the physical chemical properties of the nanocrystalline material, such as its solubility and  
15 dispersivity in aqueous buffers, water-miscible organic solvents (like DMSO and DMF) and organic solvents. In addition, the availability of pendant reactive groups such as -NH<sub>2</sub>, -COOH, and -SH is important in covalent coupling of the nanocrystal to either solid phases or ligands of analytical value. The capping process confers reagent-like properties on the  
20 nanostructured material so that it may be handled as any chemical or molecule.

Numerous methods exist for the synthesis of inorganic phosphors. The most recent approaches invoke vacuum deposition and inkjet printing technology and combinatorial chemistry. Symyx Technologies (Sunnyvale  
25 CA) and Lawrence-Berkeley National Laboratories (Berkeley CA) have synthesized combinatorial libraries of phosphors by thermolysis of organometallic precursors on planar alumina surfaces. Large arrays (hundreds to thousands) of novel phosphors are formed simultaneously by multiple-source aerosol-assisted chemical vapor deposition through binary masks and  
30 by drop-on-demand inkjet printhead arrays. Extraordinarily high quantum efficiency (> 90% in the red - green, and 60% in the blue) for a doped LaGdSrAlO<sub>3</sub> host crystal phosphor, were quickly achieved as well as very narrow emission spectra for a GdTbCeAlO<sub>3</sub> composition. Although they can

identify ideal compositions, these methods do not yield the nanoparticle or biochemical "reagent" size phosphors. Nor does it seem necessary to set up an expensive vacuum deposition, laser evaporation or microwave plasma facility. Some applicable room-temperature organic and aqueous solvent, and  
5 sol-gel synthesis methods have been published as discussed below.

In one aspect of the invention, favored method which produces monodisperse nanocrystals of  $< 5\text{nm}$  uses ligands that cap crystal growth by direct coordination to the surface plane atoms. Thiophenol capping of metal chalcogenides (ZnS, ZnSe, CdS, CdSe) has been reasonably well studied in  
10 this regard [Lover T. et al. (1997) Chem. Materials 9(4):967] . In this process the nanoparticulate chalcogen is precipitated in the presence of the capping species. Alternately, larger atomic clusters are grown from molecular precursors which already incorporate the capping ligands. Thus micelle-encapsulated CdS particles capped with Thiophenol could be grown in a  
15 controlled manner by additions of  $\text{S}^{2-}$  ions to the emulsion [Steigerwald, M.L. et al. (1988) J. Amer. Chem. Soc. 110(10):3046]. An inorganic polymer-like nanostructure forms wherein the added sulfide displaces the caps, forms a new CdS layer, and the caps shuffle to the surface atomic plane. Herron et. al. [(1990) J. Amer. Chem. Soc. 112(4):1322]  
20 demonstrated that particle size could be controlled between  $15\text{\AA}$  and  $35\text{\AA}$  by a capping strategy involving the competitive reaction rates of  $\text{S}_2^{2-}$  and thiophenol with  $\text{Cd}^{2+}$  in micellar phases. The typical micellar medium for this type of synthesis is AOT (surfactant bis(2-ethylhexyl)sulfosuccinate disodium salt, AKA dioctylsulfosuccinate sodium salt). A representative detailed synthesis  
25 for thiophenol capped ZnS and CdSe can be found in Kortan, A.R. et. al. (1988) J. Amer. Chem. Soc. 112(4):1327.

The above method, and the others described below, require that inert atmosphere ( $\text{N}_2$  or Ar), and deoxygenated solvents be used throughout the synthesis.

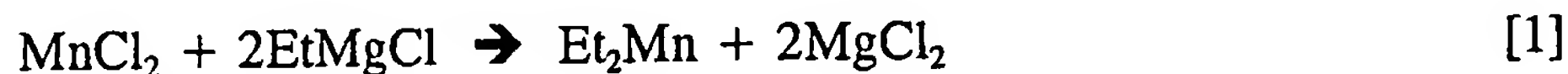
30 Several important variations to the strategies for nanoparticle size control have been introduced. Ultrasmall CdS particles were prepared in aqueous solution by first preparing the  $\text{Cd}^{2+}$  (from  $\text{CdCl}_2$ ) in a 10X molar excess of 2-mercaptoethanol, 2-mercaptoethyamine, 3-mercaptopropionic

acid, or L-cysteine. As a parameter, pH was adjusted with HCl and NaOH. Equimolar Na<sub>2</sub>S, freshly prepared, is then added. The size of resulting clusters was determined by dynamically following the change in the band edge of the excitation spectrum with millisecond spectroscopy techniques.

5 Crystal size was dependent on the concentration of thiolate ions, and pH. Both mercaptoethanol, and cysteine capped growth forming stable, very small (<3 nm) crystals in the pH range 6 to 8. Thus accessible reactive groups suitable for "peptide" bond formation were introduced in the capping process.

10 The above discussion considers synthesis of II-VI semiconductor "host" crystals. These generally have a broad emission spectrum which would have considerable overlap between CdS and ZnS, as an example. Taken on their own, these undoped materials would not give any better spectral separation than conventional organic fluorophors such as fluorescein  
15 and rhodamine. However, when they are doped to less than 5% with a Lanthanide atom (Ce, Dy, Sm Eu, Tb) or Mn, conduction band carriers (electrons) are trapped to the dopant atom and recombine with valence band carriers (holes) through the dopant's radiative and non-radiative transitions. The emission spectrum becomes dominantly that of the dopant. And, in the  
20 case of Lanthanides, this is characteristically narrow band. The doped Zinc Sulfide and Yttria nanocrystals, which are equivalent to Lanthanide chelates in their narrow band, non-overlapping emission spectra are most appropriate for our assay purposes. ZnS:Mn, was first described in detail, and characterized [Bharagava R.N. Doped nanocrystalline materials - Physics and  
25 applications. J. Luminescence 70: 85 - 94, 1996.].

Diethyl manganese is first prepared via a Grignard reaction (scheme [1]).

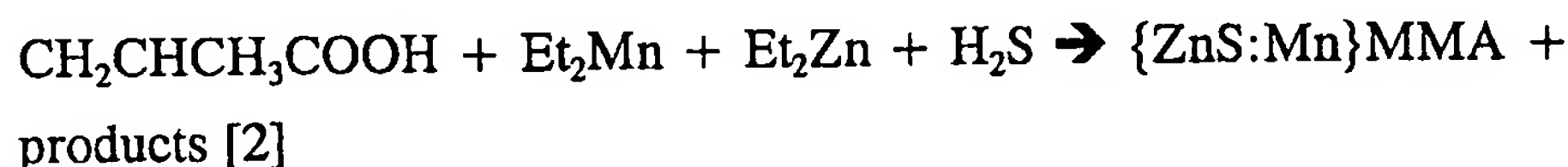


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Although it does not completely dissolve, 150 mg of the MnCl<sub>2</sub> is dispersed in THF to give sufficient Et<sub>2</sub>Mn partial product for the subsequent doping of ZnS. To this is added 69 ml of a stock 2M solution of the diethylmagnesium chloride (Aldrich) corresponding to a 13.8 micromolar

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solution. The color of the solution is observed during the reaction - progressing from colorless before addition, to yellow upon addition, then to orange, and after a few minutes to brown. The critical time for transfer of this partial product into the second Grignard reaction (scheme [2]) is during  
5 the orange coloration phase.



10 In this second reaction the receiving solution is 0.5 ml methacrylic acid plus 4.1 ml of a 1.1 M stock solution of diethyl zinc (Aldrich) (4.5 millimolar) in 110 ml of toluene. The yield is relatively low. All procedures use a Schlenk line, degassed reagents and nitrogen atmosphere. Hydrogen sulfide saturated toluene was prepared separately by purging H<sub>2</sub>S gas through toluene in a dual  
15 septum wash bottle. 23.8 ml of this was taken into a 50 ml glass syringe and rapidly injected into the second solution. The resulting methacrylic acid-capped manganese-doped zinc sulfide nanocrystals are centrifuged out, and washed by resuspension and centrifugation in THF, butanone and acetone.

Modifications to the above scheme include substitution of dibutyl or  
20 dimethyl manganese for diethyl manganese - these being formed by reaction of MnCl<sub>2</sub> with butyl lithium or methyl lithium. Oleic acid and polymethylmethacrylate were also suitable as capping species.

Bhargava has also reported having synthesized ZnS:Tb, ZnS:Eu, Y<sub>2</sub>O<sub>3</sub>:Tb and Y<sub>2</sub>O<sub>3</sub>:Eu. However, the details of synthesis are not disclosed  
25 other than that the lanthanide doped ZnS follows the above method, and that sol-gel techniques are applied to the doped Yttria synthesis.

These host crystals doped with Tb and Eu provide the non-overlapping emission spectra characteristics which would be suitable for dual  
simultaneous analyte measurement. The methacrylic acid cap/surfactant  
30 prevents aggregation while its -COOH groups are potentially available for grafting to other molecules of interest (eg. B12 and pteric acid).



Counio et. al. [Proc. Electrochem. Soc. (1997) 97-1:492] present a relatively straightforward synthesis route for CdS:Mn which prevents superlattice formation, and yields uniform size nanocrystallites as a pure powder, as colloidal suspension in an organic solvent, or as a mixed organic-inorganic silica matrix sol-gel. In the method, two solutions of Cd and Mn Acetates, and Na<sub>2</sub>S are dissolved in separate aliquots of Ethylene Glycol. The yellow precipitate formed by mixing the three solutions in the molar ratio Cd<sub>x</sub>Mn<sub>(1-x)</sub>S (0.96 < X < 0.99) is washed with methanol and suspended in triethylphosphate. The suspension is heated at 215°C (the boiling point of triethylphosphate) under N<sub>2</sub> to yield a yellow-orange colloidal solution. The particles are precipitated by addition of heptane. This powder may then be redispersed in a pyridine solution of an alkoxysilane, without formation of aggregates, to prepare a sol-gel.

A similarly uncomplicated method for preparing ZnS:Mn is available. Here also the metal acetates, Zn(CH<sub>3</sub>COO)<sub>2</sub> and Mn(CH<sub>3</sub>COO)<sub>2</sub>, (molar ratio 100:1) were dissolved in methanol, and H<sub>2</sub>S gas was passed into the solution under vigorous stirring. As in the case of CdS the size of the resultant nanocrystallites could be controlled by the molar concentration of Zn<sup>2+</sup> in the solution - 10<sup>-3</sup> M producing particles of about 3.5 nm diameter. An equal concentration of (poly)methylmethacrylate (PMMA) was added to the solution as a surfactant. PMMA "passivation" enhanced the photoluminescence (PL) efficiency by a factor of 4 over the unpassivated nanoparticle. The "radiation-induced luminescence enhancement" (RILE) effect was also observed for the PMMA-coated particles. That is, the luminescence intensity of PMMA-coated particles will decrease by an order of magnitude if aged in air for 2 months. However, after exposure to high intensity UV irradiation, the PL efficiency recovers and increases. In studying 2 - 3 nm Mn-doped ZnS nanocrystals prepared in much the same manner, Isobe et. al. [Materials Research Society Symposium Proceedings (1997) 452:305] compared the relative photoluminescence enhancement of methacrylic acid and methylmethacrylate capping monomers and HCl. The acid environment of HCL and methacrylic acid was found to reduce the incorporation of Mn into the nanocrystal by preferential ion exchange or



dissolution of the Mn over Zn. Reduction of the incorporation of Mn, possibly down to a single atom, in the acid environment gave 8 to 10 times higher luminescence than in methylmethacrylate. "Baking" the methacrylic acid-capped nanocrystalline powders may also be used to polymerize the monomer, and further enhanced the luminescence by a factor of 10. XPS and IR analyses revealed a bonding between the crystal S and O of the carboxyl. Energy transfer from the methacrylic acid to the host crystal was also strongly suspected. Bowen Katari et. al., [J. Phys. Chem. (1994) 98(15):4109] studied CdSe nanocrystalline surfaces by XPS, and determined that a monolayer of oxygen is rapidly physisorbed to freshly prepared clean surfaces. The adsorbed oxygen may proceed to break chalcogen crystalline bonds forming SeO. It is possible that the UV treatment photo-ionizes one or both of the surface oxygen atoms and the PMMA inducing them to react with each other, and permitting the crystal bond to reform.

As described above, ZnS or CdS can be capped with thiophenol and Kortan et. al. Preferred ligands which incorporate both a mercapto, or thio- (sulfide) group for host crystal surface plane co-ordination, and as a second functional group amino-, hydrazide-, carboxyl-, or hydroxyl- group for conjugation to proteins etc. Additional ligands include the following (all are available from Aldrich):

- thioanisole (methyl phenyl sulfide)
- S-(thiobenzoyl) thioglycolic acid
- thiocamphor
- 4,4'-thiobisbenzenethiol
- thiocholesterol
- 3-(thiocarbamoyl) dithiocarbazic acid (sodium salt)
- thiocarbohydrazide
- thiourea
- thiosemicarbazide
- thiosalicylic acid (2-mercaptobenzoic acid)
- 2-thioxo-4-thiazolidinecarboxylic acid
- thiodiglycolic acid (thiodiacetic acid)
- thiodiphenol

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- 3,3'-thiopropionic acid
- 5-thio D-glucose
- thiolacetic acid
- mercaptosuccinic acid (thiomalic acid)
- 5 mercaptonicotinic acid
- 2-mercaptoethylsulfide
- 2-mercaptoethylamine
- 3-mercaptopropionic acid
- 3-mercapto-1-propanol
- 10 thioctic acid (6,8-dithiooctanoic acid)
- thiodianiline
- 2,2'-thiodiethanol
- 6-mercaptopurine riboside
- 2-thiocytidine dihydrate
- 15 4-thiouridine

The size of the nanoparticle is advantageously between 10 Å and 1000 Å diameter. In addition to the use of phosphor nanoparticles in immunoassays, the phosphorescent nanoparticle-coupled nucleotide substrates  
20 of the present invention find application in DNA:RNA sequencing, hybridization, and other analytical and synthesis procedures associated with gene mapping and expression work.

Gold colloidal nanoparticles attached to thiol-modified nucleotides have been reported. The gold-sulfur bond is well known to be a strong  
25 coupling and has been used for the preparation of many alkane-thiol-gold structures in the fields of microelectronic materials and electron microscopy/cell ultrastructure imaging. This differs from our application in that the nanoparticle is not a phosphor, and that it depends upon the gold-sulfur coupling mechanism.

30 DNA has been attached to mercaptoethylamine (MEA) capped CdS nanoparticles [Murphy, C.J. et al. (1997) Material Research Society Symposium Proceedings 452:597]. However, the DNA was not covalently bound to the nanoparticle. The MEA serves to create a protein-like (-NH<sub>2</sub>)

surface for studies of DNA structure. Phosphorescence was decreased by “kinked” but not “linear” sequences. The intent of this work however, was not toward labeling a nucleotide for hybridization or sequencing purposes as is described in this application. Linear and circular sequences of (plasmid) DNA have also been used as templates for CdS nanocrystalline phosphors. In this instance however, the DNA ultrastructure in solution served to nucleate the formation of the crystal due to  $\text{Cd}^{2+}$  ion interaction with the DNA sequence. Electron microscope images revealed approximately 5 nm crystallites in string-of-pearls formation along linear or circular strands of the DNA matrix. In this case also, the purpose is structural investigation. The mechanism is non-specific nucleation of metal ion on nucleic acid polymer structures, not covalent conjugation of a different color of phosphor nanoparticle to each of the 4 nucleic acids of DNA/RNA for the purpose of determining sequence or hybridization and mapping.

Phosphor labelled reporter particles may be used to detect specific DNA/RNA nucleotide sequences. The present invention discloses DNA:RNA nucleotide substrates incorporating a spacer/linker molecule and nanoparticle phosphor attached thereto. The DNA:RNA nucleotide is composed of a ribosyl, deoxyribosyl or dideoxyribosyl moiety having a derivatized purine or pyrimidine attached at its 1' position. The spacer/linker is introduced at the 4 or 5 position of the pyrimidine, and the 6 or 7 position of the purine moiety. The spacer/linker is terminated at the end opposite the nucleotide binding site with a functional group suitable for covalent binding to the phosphorescent nanoparticle. Functional groups include, but are not limited to, amino, carboxy, thiol, hydroxy, epoxy, N-hydroxysuccinimide ester, and maleimido groups. The spacer/linker functional group is coupled to like functional groups on the surface of a nanoparticle phosphor by means of bifunctional or heterofunctional crosslinkers, (di)aldehyde, carbodiimide and other known methods. Such a spacer/linker distances the phosphor nanoparticle from the hydrogen bonding region of the complementary purines and pyrimidines in the assembly or digestion of double-stranded DNA or DNA:RNA by the polymerase, transcriptase, and other DNA/RNA-modifying enzymes. Steric hindrance and interference with enzyme

processing of the substrate, and quenching of phosphorescence are thus reduced. In one embodiment of the invention, an oligonucleotide complementary to all DNA or RNA sequences to be screened is covalently attached to a metal oxide surface via a silane linker. The DNA/RNA sample  
5 is then introduced and will bind to the oligonucleotide on the test surface.

A tissue or sequence specific RNA or DNA transcript labelled with the phosphor nanoparticle is applied and the binding of the labelled moiety indicates the presence of the target sequence. Variations of this technique may be used to quantify the amount of nucleic acid detected or to screen, in  
10 an array format, libraries for the presence of a specific sequence. In a further embodiment, phosphor nanoparticles may be used as colour emitting reagents for DNA/RNA sequencing. This sequencing method is based on established dideoxy terminator technology but uses a novel labelling technique.

15 Nucleotides are labelled with phosphor nanoparticles as essentially described above. The dideoxy terminator corresponding to each nucleoside is labelled with a phosphor nanoparticle intentionally doped with an impurity such as described above that will emit radiation in narrow, non-overlapping regions of the spectrum such that transcripts of increasing size can be  
20 identified by a different color based on, at which nucleotide base the sequence terminates. In one aspect of the invention, the target DNA forms a template for a polymerase chain reaction incorporating a mixture of nucleosides and dideoxy terminators labelled as described above. The resultant transcripts of varying lengths are separated by polyacrylimide gel electrophoresis. The gel  
25 can then be scanned by the apparatus to be described with respect to Figure 18 and the resultant phosphorescent signature can be analyzed to provide a read-out of the DNA sequence.

DNA/RNA substrates labelled with phosphor nanoparticles designed to emit radiation of varying wavelengths may also be used to detect specific  
30 sequences in a genomic library array such as shown in Figure 17.

Phosphorescent particles doped to express different colours can be used to detect the presence or absence of or even relative amounts of several constituents in a mixture. For example, this could provide a rapid means for

detecting several contaminants in a product in a single step or for detection of several infectious agents in a single sample.

An approach to generating particulate phosphor reporters is via adhesive binding of streptavidin or antibodies to the crystalline phosphor as taught by Tanke et. al. in U.S. Patent 5,043,265. However, such adhesive binding is not stable under a wide range of buffer conditions. We have covalently caged or crosslinked enzyme-labelled monoclonal antibodies and avidin to similar micron size phosphors (ZnS: Cu:Al, ZnS: Ag, and Yttrium Oxysulfide:Fu) as described in Example 1. Attention is given to the pH and buffer concentration characteristics of the adhesive binding. Other particle-protein studies suggest the optimum adhesion occurs at the isoelectric point of the protein.

A category of viruses is the phage and in particular the bacterial phage. In accordance with the technology described in U.S. Patent 5,403,484, Ladner et al. provide phage particles which can express on their surface engineered binding domains for target molecules. The target molecules may be biological or synthetic macromolecules as well as other organic and inorganic substances. The phage particles by virtue of their protein structure have dielectric properties. Phage particles are commonly of nanometre size usually in the range of 10 to 30 nm. Phage particles may be selected which have the desired dielectric constant usually of about 3, for use in this invention as reporter particles in a capacitance biosensor. Considering that water has a dielectric constant about 81, the dielectric constant of a phage renders it quite useful in this invention. Phages behave as very high molecular weight proteins in solution and therefore can be caused to move in an electrophoretic field - this being advantageous for separation from the surface of the capacitor. In addition to the phage particles being engineered to express on their surface desired binding domains, the phage particles may also include DNA sequence information which when expressed provide reporter material on the surface of the phage, for example, protein, such as phycoallocyanine, which may have fluorescent properties. Hence, the phage particles may be used in the assay system as a bifunctional particle. The phage particles may also be engineered to express binding domains, such as



the iron binding protein apoferritin, which would render the phage particles magnetic. The phage particles can therefore be used as particle reporters either as particles having inherent dielectric properties or engineered to have one or more of dielectric properties, paramagnetic properties and  
5 phosphorescent properties.

The phage particles can be disrupted, their nucleic acid material removed, and then repackaged around a solid-phase phosphor dielectric and/or paramagnetic nanoparticle by the methods generally outlined in Kossovsky's U.S. Patent 5,219,577.

10 As stated above, phage particles can be engineered to express large amounts of foreign DNA and as such they have frequently been used for the screening of genomic libraries.

Thus the use of phage particles which, can incorporate foreign DNA and can also express a suitable label for detection, as described above,  
15 provides for a significantly enhanced screening method. For example, an array comprising various aspects to be screened for can be reacted with a phage population expressing genomic fragments and a phosphorescent marker. Analysis of the phosphorescence in the grid indicates to which moiety the phage is binding. Thus one can rapidly and safely detect specific  
20 binding to a target molecule. It is understood that while the descriptions above focus on phage as phosphorescent particles, the same techniques may be applied using nucleic acids derived from phage technology or elsewhere, antibodies, or other recognition molecules directly packaged around a phosphor nanoparticle. An advantage of the phage technology is that they are  
25 easy to produce and do not require extensive purification schemes.

Phage particles can also provide an amplification step in immunoassays by virtue of their potential bifunctionality. For example, a phage particle could be engineered to express a sequence of interest and to also express a recognition site (e.g. enzyme binding, avidin) for a labelled second reagent  
30 such as an enzyme or biotin.

A significant advantage in using engineered phage particles is that the phage particles, in expressing binding domains on their surface to target molecules, can then be designed to bind to the targeted analyte, the

recognition molecules or the analyte entities. Phage particles inherently provide the nanometre size along with the engineered ability to present binding domains which effectively replace any steps needed in respect of other types of nanoparticles in linking the phage particles to the recognition molecules, target analyte or analyte entities. Considerable flexibility is provided in use of phage particles in designing the assay whether it be competitive, sandwich, direct or indirect type of assay. It is also understood that the phage particles may be used in combination with the other types of nanoparticles to provide more than one system in evaluating presence or absence of the reporter phage particles.

It is also possible to select micron and nano size particles which have two or more of the dielectric properties, paramagnetism and phosphorescence. Such combination of properties in the particles may be advantageous in providing a cross-check of the biosensors sensitivity and operation.

Certain theoretical considerations are necessary for discussing in detail the capacitor sensors as shown in the Figures. The capacitance of a parallel plate capacitor in which the region between the plates is filled with vacuum (free space) is

20

$$C = Q/V = A * \epsilon_0/d \quad (1)$$

where Q is the charge in Coulombs, V is the voltage between the plates, A is the area of the plates in square metres, and d is the distance between the plates in metres, and  $\epsilon_0$  is the permittivity of free space ( $8.85 \times 10^{-12}$  coulomb<sup>2</sup>/Newton-Metre<sup>2</sup>).

25

For capacitors in series the total capacitance is expressed as

30

$$1/C = 1/C_1 + 1/C_2 + \dots + 1/C_n \quad (2)$$

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Matter is polarized to varying degrees such that the permittivity ( $\epsilon$ ) is a multiple of that of vacuum ( $\epsilon_0$ ).

$$\epsilon = K * \epsilon_0 \quad \text{or} \quad K = \epsilon/\epsilon_0 \quad (3)$$

5

Where K is the dielectric constant.

$$C = (\sigma_f * A)/(E * d) \quad (4)$$

10 Where  $\sigma_f$  is the charge density ( $Q_f/A$ ) of free space, E is the applied electric field, and d is the distance between the parallel plate conductors.

$$E = (\sigma_f + \sigma_p)/\epsilon_0 \quad (5)$$

15 Where  $\sigma_p$  is the polarization charge density due to the electric dipole charge displacement.

$$\sigma_f = \epsilon_0 * (1 + \chi) * E \quad (6)$$

$$P = \epsilon_0 * \chi * E \quad (7)$$

20

Where P is the dielectric polarization field, and  $\chi$  is the dielectric susceptibility of the material.

$$C = (\epsilon_0 * (1 + \chi) * A)/d \quad (8)$$

25

$$K = (1 + \chi) \quad (9)$$

$$C = (\epsilon_0 * K * A)/d \quad (10)$$

Equation 10 relates the capacitance (C) surface area (A), and separation distance (d) of the "plates" of the parallel plate capacitor model, and to the dielectric constant (K) of the material filling the space between the plates.

30

According to equation 2, if the space between the plates of the capacitor is filled with two different dielectric materials having thickness  $d_1$  and  $d_2$  respectively and dielectric permittivities of  $\epsilon_1$  and  $\epsilon_2$  respectively, then the capacitance is expressed as:

5

$$C = \epsilon_0 * A / (d_1/\epsilon_1 + d_2/\epsilon_2) \quad (11)$$

Both Newman U.S. Patent 4,822,566 and Bataillard [Bataillard et al., Anal. Chem. (60):2374-2379, 1988] relate measurement of capacitance  
10 change to the change in effective dielectric constant, and effective thickness of the dielectric layer upon complex formation between the analyte and the recognition molecule. This theory is recast in terms of dielectric particles for the purposes of the present invention. Functionally, water either occupies or is displaced from a layer whose thickness is determined by the effective  
15 diameter of the dielectric particles - including the linker and attached analyte or recognition molecules. If the dielectric particle itself has a diameter much larger than that of the bound molecules (as would be the case for such drugs as phenytoin, digoxin, cyclosporin and FK-506), then the dielectric constant of the particle makes the dominant contribution to the measured change in  
20 capacitance. If the attached molecules and the particle have similar diameters, then the effective dielectric constant will lie between that of the attached molecules and that of the particle. Finally, if the attached molecules have a much greater diameter than that of the particle, then the dielectric constant of the molecules will make the dominant contribution to the  
25 measured change in capacitance.

For the purposes of the following discussion it is assumed that water is by far the most abundant species in a sample, and that its dielectric constant dominates the net dielectric constant of the sample. In real samples, high protein and lipid species concentrations may shift the net dielectric constant of  
30 the undiluted sample toward the value of 1 by an unknown amount.

The "plates" or metallizations forming the plates of the capacitor are covered with a water- and electrolyte-impermeable passivation layer of thickness  $d_p$  and dielectric constant  $K_p$ . The two layer dielectric model of

equation 11 is therefore applied. The analyte and recognition molecule then form a complexing layer attached to this passivation layer, dielectric particles being either added to or displaced from this layer. If water (subscript w) exchanges with a dielectric layer (subscript d), the change in capacitance is

5 described by the two conditions:

$$1/C_1 = (d_p/K_p + d_d/K_d)/(\epsilon_0 * A) \quad (12A)$$

$$1/C_2 = (d_d/K_p + d_d/K_w)/(\epsilon_o^* A) \quad (12B)$$

10	Where $C_d$	=	Capacitance of the complexing layer with dielectric particles
	$C_w$	=	Capacitance of the complexing layer with water in place of particles
	$d_d$	=	Effective thickness of the dielectric layer (approximately the diameter of the dielectric particles for small analysis and recognition molecules)
15			
	$d_p$	=	Thickness of the passivation layer over conductors (plates) of the capacitor (eg. SiN + SiO <sub>2</sub> )
20	$A$	=	Area of capacitor plates
	$K_d$	=	Dielectric constant of particles
	$K_w$	=	Dielectric constant of water
	$K_p$	=	Dielectric constant of passivation layer

The thickness of the water layer is the same as that of the dielectric particle layer with which it exchanges. Thus it can be seen from equations 12A and 12B that the change is determined exclusively by the change in dielectric constant  $K_d$  to  $K_w$  in the complexing layer. It is also seen, that for a capacitive sensor with a given plate area ( $A$ ), the sensitivity is increased by having a passivation layer with high dielectric constant ( $K_p$ ), and minimal thickness ( $d_p$ ), to make the term  $d_p/K_p$  as close to zero as possible. In practice, defect or pinhole-free passivation layers of silicon nitride must be more than about 100 nm thick. Polymer coatings, such as parylene or



polyimide, may be 1 micron or more in thickness to guarantee pinhole-free surfaces. Overlaid upon these is a  $\text{SiO}_2$  layer of approximately 100 nm thickness to provide for covalent immobilization via an aminoalkylsilyl or thiolalkylsilyl linker. The resulting passivation layer may therefore be  
5 expected to be about 300 nm or more in thickness in practical devices.

To optimize the capacitive sensor device design, various practical values were substituted into equation 12 for the parameters  $d_p$ ,  $d_d$ ,  $K_p$ ,  $K_d$  and  $K_w$ . The model calculates the maximum expected change in capacitance which would occur if a dense dielectric layer of nanoparticles 300 nm in  
10 diameter (thick) exchanged with a water layer 300 nm thick. The maximum  $\Delta C$  is achieved with the thinnest possible passivation layer having the highest possible dielectric constant (eg. a ferroelectric ceramic such as  $\text{BaTiO}_3$ ), and dielectric nanoparticles having a dielectric constant as close as possible to 1.

15

The advantage of this invention is that:

1. the dielectric constant of the label/tag is chosen to be optimally different from that of the buffer medium to effect the maximum  
20 capacitance/permittivity change of each molecule exchanged/displaced.
2. the dielectric label/tag may be made arbitrarily large relative to the analyte or recognition molecule such that one can control the  
25 "quantum" change in measured capacitance for each molecular recognition or binding event. That is each label/tag particle can be made to exchange with an arbitrarily large number of buffer molecules.
- 30 3. the dielectric label/tag can be selected to contribute the dominant dielectric constant in the complex (analyte-recognition molecule-dielectric label/tag) thereby minimizing the analyte-to-

analyte, assay-to-assay variation which is inherent in the analyte-recognition complexes themselves.

This permits more uniform instrumental design, assay chemistry  
5 and sensor device manufacturing processes.

In accordance with one aspect of the invention, a test component of a capacitance biosensor for use in a suitable diagnostic apparatus is shown in Figures 3 and 4. The test component 50 has a test surface 52 with capacitor electrodes 54 and 56 along the length of the test surface where the electrodes  
10 54 and 56 may be oppositely charged through circuit contacts 58 and 60. The electrodes and circuit contacts may be laid down on a quartz, i.e., silica substrate 62 where they may be deposited by chemical vapour deposition on a grown layer of silica on a silicon wafer. The electrodes may be of titanium plus or minus gold metallization layer etched to produce the electrodes or  
15 plates of the capacitor thereby achieving a large face area with interdigitating structure. Laid over top of the metallization layer is a passivation layer which may be of silicon nitride. The passivation layer is a pin hole defect-free dielectric passivation layer which may be approximately 300 nm thick. The dielectric passivation layer as laid over the metal plates prevents sample  
20 and reagents from permeating to or reacting with the electrodes. As shown in Figure 4, the dielectric passivation layer is indicated at 64. On top of the passivation layer is a further dielectric layer which may be, for example, of silicon dioxide. The layer 66 may be approximately 200 nm thick. The purpose of the outer silicon dioxide layer is to facilitate linking of the  
25 recognition molecules or other bioactive molecules to the surface. The layer 66 is not an integral requirement of the capacitance biosensor other than to provide this surface for covalent linking or other type of linking or mobilization of the bioactive molecules to the surface of the sensor. As described in Newman U.S. Patent 4,822,566, the linker molecule as attached  
30 to the outer SiO<sub>2</sub> layer 66 may be designed to space the bioactive molecules away from the electric double layer of the sensor to enhance the base and test signals of the device. An advantage of the first and second dielectric layers

64 and 66 which has not been apparent in prior biosensors, is that these layers have the ability to amplify or attenuate the sensitivity of the device. Layers 64 and 66 should be as thin as possible for best sensitivity. A ferroelectric layer of barium titanate may be much thicker and still provide high sensitivity. Elimination of the outer dielectric layer may increase sensitivity. Other considerations include particle diameter varying sensitivity. The particle dielectric constant should be as different from that of the sample buffer as possible. Preferably the dielectric particle has a dielectric constant K as close to one as possible rather than a very high dielectric constant K such as in a ferroelectric particle.

Figure 12 shows how capacitance and percent change in capacitance (sensitivity) are related to particle diameter. A thin film device having 300 nm of  $\text{Si}_3\text{N}_4$  ( $K=8$ ) plus 200 nm  $\text{SiO}_2$  ( $K=6$ ) has about 50% higher sensitivity than a thick film device with 25000 nm of  $\text{BaTiO}_3$  ( $K=4500$ ) and 1000 nm of  $\text{SiO}_2$ . Figure 13 shows how replacing the  $\text{Si}_3\text{N}_4$  of the thin film device with  $\text{BaTiO}_3$  and removing the glass layer of each of the thin and thick film devices improves sensitivity again, the thick film device becoming equivalent to the thin film device. An important consideration for a single thin or thick layer of  $\text{BaTiO}_3$  is that the layer is passive, and impermeable to sample, and capable of derivatization. Figure 14 shows these effects again for a thin film structure with coplanar interdigitated capacitor "plates".

The advantage of nanoparticle-tagged reporters over the unaided molecular complex detection approach of previous work is better uniformity of method for different analytes. Because there is a large "quantized" dielectric change per particle bound to or removed from between the "plates" of the capacitor, calibration and test formatting is less influenced by the great variation in molecular dimensions between steroid hormones and immunoglobulins for example. One should choose particle size to give the highest sensitivity while at the same time retaining the ability of the particles to be dispersed and interact homogeneously with the sample analyte.

Methods for forming thin and thick films of ferroelectric materials at low temperature have been improved steadily. Much of the work in the field was reviewed at the 1996 Spring Materials Research Society Meeting (April

7-12, 1996, San Francisco Marriott). Known high dielectric constant inorganic titanates, niobates, and ferroelectric polymers can be formed by many processes including low temperature chemical vapour deposition, laser photo-ablation deposition, sol-gel processes, RF magnetron sputtering, screen printing/and firing, and spin coating (in the case of the polymer). The more common electronic materials are the BST (Barium-Strontium-Titanate), PZT (Lead-Zirconium-Titanate), and lead niobate groups. It is thus possible in the case of  $\text{BaTiO}_3$ , for example, to screen print a low firing temperature paste compatible with 70Ag/30Pd metallization forming 10 to 25 micron layers on thick film substrates (K-4400 X7R, MRA Laboratories, North Adams, MA). This gives thick film devices a performance equivalent to integrated circuit or thin film devices. In addition, it is possible to form printed circuit traces on 25 micron copper-clad laminate flexible films or injection molded devices of Kapton (polymer). These may also be overlaid at low temperature with ferroelectric BST and PZT films and glass passivation layers.

Other recently developed electronic materials processes may advantageously apply to the fabrication of capacitive biosensors. For example, photo-ablative Eximer, YAG and  $\text{CO}_2$  laser etching and micromachining of metal-clad plastic laminates, ceramic ( $\text{Al}_2\text{O}_3$ ) substrates, conventional PC board materials, polyimide, and conformal coating resins are available (e.g. Lumonics Inc., Ottawa, Ontario, Canada). Sharp features can be maintained at dimensions compatible with thick film (channels 25 microns deep by 50 microns wide). Additional laser processes include package welding, mold machining, and semiconductor/ceramic film deposition and annealing.

Figure 5 demonstrates the manner in which the test component of Figure 3 may be adapted for sample injection. The test component 50 may have along each side spacer elements 68 to support above the test surface 52, a second plate 70. The element 68 may be of nominal size, such as 25 microns to define a capillary space which attracts the sample to within the device so that the presence or absence of analyte may be detected across the face of the test surface 52.

Figure 6 is a section through an alternative structure for the test component of the biosensor. The test component 72 has a central silicon substrate 74 with grown silica layers 76 and 78 on opposite surfaces of the substrate 74. As with the device of Figure 3, spaced apart electrodes 80 and 82 which may be oppositely charged and 84 and 86 on the other side of the device which may be oppositely charged. The electrodes are formed to be raised relative to the surface of silica layers 76 and 78 to define mini-wells or channels. The electrodes and mini-wells are covered with the dielectric layer of, for example, barium titanate or silicon nitride 90. On top of layer 90 is the outer layer 92 of silica which facilitates covalent linking to the desired molecules and optionally to increase sensitivity of the device. This outermost layer is 92. Suitable metal contacts are provided in the test component to oppositely charge the electrode sets 80 and 82 and 84 and 86 along the width and length of the test surface.

A significant advantage of the dual sided device is to provide a reference or control in conducting the test. As shown in Figure 7, the test component 72 may be gripped at its end portion 94 by a robotic gripper device 96. The robotic gripper device may include electrodes 98 and 100 for power supply and signal detection as desired the electrodes 80 and 82 and 84 and 86 on each side of the device. The sample 102 to be tested is contained within a sample containing semimicro-cuvette 104 where readings may be taken for the base signal and the test signal and depending upon formations on the opposite sides, provide an accurate quantitative answer for the determination of analyte in the sample.

The capacitor biosensor could be fabricated in silicon, by thin film techniques. It would appear essentially the same and have approximately the same surface area if it were fabricated by either printed circuit or thick film techniques. A double-sided structure maintains intimate thermal contact between active devices. One side is the reference device in a differential sensor arrangement so that temperature-induced noise or background is minimized. A commercial 3-axis pipetting robot (Diamedix, Tecan and Beckman are manufacturers of such sample processing robots) with a solenoid-actuated gripper precisely positions the sensor, and makes circuit



contact. Preamplifiers and other components may be incorporated into the gripper if required to improve signal-to-noise ratio.

All three manufacturing approaches require printing, or deposition of a high dielectric constant ferroelectric material such as BaTiO<sub>3</sub> over the metallization layer, and optionally, depositing silica or silicon oxynitride over the ferroelectric.

Figure 8 is an enlarged view of a proposed competition reaction using the device of Figure 6. In Figure 8 the quartz substrate 74 has the electrodes 80 and 82 provided thereon of oppositely charged polarity and the dielectric layer 90 is covered with the linking layer 92. Linking molecules 106 link the analyte entities 108 to the surface 92. Phage particles 110 have recognition molecules 112 expressed on their surface which recognize either the analyte entities 108 or the analyte 114 of the sample. When the sample is introduced to the test surface, the analyte molecules 114 compete with the analyte entities 108 for the recognition molecule site of the phage 110. This competition forms a complex of the phage particles 110 by virtue of their recognition molecules 112 binding to the analyte 114. Such competition removes the phage particles 110 from the test surface. By removal of such analyte particles from the test surface, the capacitance of the device has changed and hence, a measure of the amount of analyte in the sample can be detected by use for example, of the biosensors of Figures 10 and 11.

The sensor for sensing phosphorescence from solid-state phosphor particles may be constructed in accordance with standard phosphorescence sensing technology. The phosphorescence sensor device is comprised of a solid phase upon which is attached a primary recognition molecule which specifically complexes with the analyte to be measured. The device requires, in addition, a light source (such as a Xenon flash lamp, laser diode, LED) or pumping source (such as an electron beam, or electroluminescent mechanisms) and a sensitive light detector (such as a photomultiplier, microchannel plate, or avalanche photodiode). The method of attachment of the primary recognition molecule is preferably by covalent binding via a linker; and the solid phase may be of any material, preferably one which may be derivatized to permit the covalent binding process. The sensor/assay

reporter is comprised of phosphorescent microparticles or nanoparticles which may be covalently attached to either analyte molecules or chemical analogs of the analyte molecules or a secondary recognition molecule via a second linker. The phosphorescent micro- or nanoparticles are of water-  
5 insoluble ceramic, glass, doped-glass, inorganic or organic phosphor-loaded polymer, electro/photo luminescent bandgap semi-conductor polymers, and may have dielectric constants suitable for capacitance detection or dielectrophoretic field migration and concentration. The solid phase may take the form of a bead or particle (a carrier), a mesh, a surface providing part of  
10 a container for the sample and assay reagents, or an optical waveguide. The optical waveguide may allow source light "evanescent wave" excitation of the phosphorescent micro- or nanoparticles which have been complexed with the primary recognition molecule and held within a few wavelengths of the surface. Further, the optical waveguide may provide efficient collection of  
15 the phosphorescent light emission and focusing thereof on the phosphorescence detector. For the best signal to noise ratio, the light source will be pulsed or chopped and have a "turn-off time" much shorter than the half-life of the phosphorescence of the reporter. In addition, the detector will be blocked or electronically gated off during the ON period of the light  
20 source, then unblocked, or gated on during the OFF period of the light source.

The phosphorescence sensor exploits the trapped electron or excited states of room temperature phosphorescent materials. The delayed emission of the excited triplet state of phosphors typically ranges from microseconds to  
25 milliseconds as compared to the prompt radiative electron transition of fluorophores which takes place in typically less than 20 nanoseconds. This characteristic of phosphors permits the synchronous "gating" of the detector. That is, while the high intensity of the light source is applied to excitation of the phosphor-labelled material the light path to the detector is blocked (eg. by  
30 a chopper or other fast-acting electromechanical device) or the detector is electronically gated off (eg. grid-gated photomultipliers). Thus background light, due to source light scattered from the surfaces of the container, macromolecules or colloidal substances, and prompt fluorescence of sample

and buffer proteins and sensor device materials, is blocked from reaching the detector or contributing to the detected signal. Conversely, when the source is blocked or electronically turned off, the detector is unblocked (gated on) and can "see" only the phosphorescent signal without background contributions. This is a classical phosphorimetry method which affords the highest signal-to-noise ratio in the instrumental measurement of the fluorescence/phosphorescence.

Solid state ceramic, doped glass, semiconductor polymers and porphyrin-derivative-loaded polymeric materials may be excited by relatively inexpensive and commercially available light sources. For example, Hammamatsu manufactures long life low electronic noise Xe flashlamp modules, and SiC LEDs producing 480 nm blue light and 410 nm near UV light output have been fabricated by Sapphire Research and Production Amalgamation (Moscow, Russian Republic).

More specifically, the sensor device of Figure 9 in accordance with an embodiment of the invention, involves the evanescent wave excitation of the phosphorescent micro or nanoparticles. The test component may be of the type described with respect to Figure 2 where the recognition molecules 32 form a complex with the analyte 46 and the labelling entities 38 which carry the phosphor particle 42. The excitation radiation is projected at the waveguide 116 which may be the silica substrate. The radiation enters in the form of a direct beam 118 and travels along the waveguide 116, as indicated at 120. In accordance with evanescent wave excitation, the radiation as it travels along the surface of the waveguide 116 only excites the phosphorescent particles 42 bound to the complex of the recognition molecule 32 with the analyte 46. Hence, the particles 42 emit radiation, as indicated by lines 122, whereas the particles 42A, which are not bound to the surface and free in solution, are not affected by the radiation and hence do not phosphorescence. A suitable device as described with respect to Figure 11 is then used to detect the phosphorescence 122 and provide a quantitative determination of the amount of analyte 46 complexed with the recognition molecules 32.

Figures 10 and 11 provide block diagrams of the devices for sensing capacitance or phosphorescence. Figure 10 shows a test instrument configuration for measuring differential changes in sensor capacitance due to analyte-related addition or removal of dielectric particles between the "plates" of the capacitor sensor under test. The Wheatstone Bridge 124 circuit permits the measurement of voltage amplitude and phase differences between the active device 126 under test in one arm of the bridge between electrodes 128 and 130 and an identical capacitance device 132 with ionizing radiation-inactivated or crosslinked recognition molecules in the opposite arm of the bridge between electrodes 128 and 134. Thus the reference capacitor 132 compensates for such variables as temperature, sample protein concentration, lipemia, drugs, and other interference factors which may alter the device response to the analyte concentration.

The bridge excitation voltage, waveform, and frequency may be downloaded to the programmable function generator 136 (Stanford Research Systems DS345-01) under computer 138 software control (National Instruments Lab-Windows/CVI) via an IEEE 488.2 or GPIB interface (National Instruments PCI bus interface 777158-51). As an alternative, for sine wave excitation only, the lock-in amplifier 140 (Stanford Research Systems SR830-DSP) can be remotely programmed for bridge excitation frequencies up to 100 KHz. Input range and filter settings are downloaded to the lock-in amplifier 140 digital signal processor, and in-phase and quadrature voltage data from the bridge circuit 124 is uploaded to the computer 138 for analysis via the IEEE instrument bus. The Stanford Research Systems Application Note #3, About Lock-in Amplifiers provides details on the sensitivity of impedance detection possible with digital signal processing lock-in amplifiers.

Figure 11 shows in block form the instrumentation for measuring phosphorescence of solid-phase nanoparticles. The configuration is essentially that of a classical "phosphoroscope". A 6 mw helium cadmium laser 142 (Liconix) emitting at 340 nm wavelength is incident upon a high speed chopper wheel 144 (Stanford Research Systems SR540) which alternately passes the beam through to the sensor device 146 under test or



reflects it to a reference photodiode (Hamamatsu photodiode-amplifier module G1957 148). The reference photodiode 148 thus receives pulses of laser light and produces output signal pulses to the lock-in amplifier 150 (Stanford Research Systems SR830 DSP) which are proportional in amplitude to the intensity of the laser pulses. These output pulses provide both a phase locking signal and a means for correcting phosphorescence measurements for variations in the exciting laser light intensity. Laser pulses passing through the chopper aperture 152 excite the nanoparticles while the view from the photomultiplier detector 154 (Hamamatsu H5773) to the test device 146 is blocked by a vane 156 of the chopper. Conversely, when the laser beam is blocked by the chopper 144 phosphorescence emission from the device 146 is focused through an aperture of the chopper onto the photomultiplier 154 via a high light collection efficiency lens 158. Thus the photomultiplier 154 and photodiode 148 signals are 180° out of the phase. In addition, the photomultiplier 154 is shielded from laser source scatter from the device thus permitting more sensitive detection. The scheme is applicable only to those phosphors which have half lives on the order of milliseconds. The photomultiplier 154 module contains an integral Cockcroft-Walton high voltage multiplier whose output is set by a low input voltage provided by the programmable power supply module 160 (Xantrex XT60-1). The sensitivity or gain of the photomultiplier may therefore be set under computer 162 software control (National Instruments Lab-Windows/CVI) via the IEEE 4888.2/GPIB interface with the programmable power supply. As in the case of the capacitance sensor test system of Figure 10 input range and filter settings are downloaded to the lock-in amplifier-digital signal processor, and data from the detectors 148,154 is uploaded to the computer 162 for analysis via the same instrument bus. Stanford Research Systems Application Note #4, Signal Recovery with Photomultiplier Tubes: Photon Counting, Lock-in Detection or Boxer Averaging provides further information on the sensitivity of photomultiplier detection using lock-in amplifiers.

An alternative device for measuring phosphorescent emissions from phosphor labels is shown in Figure 15. The apparatus generally designated 170 has a test substrate 172 in this embodiment. The test substrate has a



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glass or plastic base layer 174 which is coated at 176 with an electrically conductive indium tin oxide thin film. This coating renders the test substrate transparent to the phosphorescent emissions from the phosphorescent label 178. The label 178 is associated with a recognition molecule 180 which is bound in this embodiment to the test analyte 182 which is fixed to the surface of the thin film 176. The electron beam generating device 184 comprises two sets of electrodes 186 and 188 spaced apart by a dielectric material 190. An RF generating device 192 generates a pulse signal which is in turn develops an electric field in region 194. A glow discharge occurs when an RF signal of suitable voltage is applied between electrodes 186 and 188. A field electrode 196 is forward of electrode 188 and is charged several hundred volts relative to the charge on electrode 188 to control and focus the emission of charged particles from region 194. The electrode 196 has an aperture 198 through which the electrons flow towards the oppositely charged test substrate 172. The charge on the substrate is of a sufficient magnitude relative to the charge on electrodes 188 and 196 to provide an electron accelerating potential in providing a particle accelerating field for particles to flow in the focused beam 200 towards the phosphor labels which are bound or fixed, relative to the test substrate 172. The electron beam causes the phosphor particles to phosphoresce. The phosphorescent radiation travels through the test substrate 172 in a diverging envelope 202 which is focused by lens 204 towards to photomultiplier detector 206 for detecting the extent of phosphorescence from the labels 178.

The technology for developing the electron beam may be of the type described in U.S. Patent 5,450,103 which was commonly used in electrographic printing systems. The electron beam generating devices may be fabricated in a serial manner in thin film semiconductor material. The device may be used in air and is normally spaced less than 1 mm from the target and preferably in the range of about 0.1 to 0.3 mm and most preferably 0.2 mm from the target. Surprisingly, the use of this electron beam positioned in such close proximity to the test substrate allows the use of an electron beam in air to excite the phosphorescent particles. In the past it has been thought that phosphor particles would normally be excited to

phosphoresce in a vacuum if an electron beam is used to excite the phosphor material.

With reference to Figure 16, an enlarged view of the surface of the test substrate 172 is shown. The thin layer 176 of the indium thin oxide is approximately about 30 to 300 nm thick and is bonded to the glass or plastic substrate 174. The metal oxide 208 of the indium thin oxide layer is derivatized with a silane spacer 210 so as to covalently link, for example, either the amino terminus of a protein or amino-linked polynucleotide sequence. The capture oligo 212 is then brought into contact with the sample of DNA material to be analyzed. The capture oligo recognizes the analyte in the form of a DNA sequence 214. The labelled recognition molecule 216 has the phosphor label 218 attached thereto. The phosphor particle is cause to then phosphoresce when excited by the electron beam.

Figure 17 illustrates the use of a two-dimensional array of individual electron beam generating devices 220. The array is set up in the x (columns) and y (rows) axis which can be individually switched on in a controlled manner, as demonstrated by beam generating device 222 projecting the electron beam 224 at a test substrate 226. In form, the elements of the array are addressable in the same way a field emission display device would address individual pixels of the flat panel display. In accordance with this embodiment, the test substrate is also set up in a two-dimensional array to provide a plurality of test substrates 226. This provides for multiple assays in a single setup. Again, the test substrates would be transparent to the phosphorescence 228 which is focused by a fresnel lens 230 on a photomultiplier tube 232. The photomultiplier tube may have an optical interference filter wheel 234 so that the photomultiplier tube only detects the phosphorescence of a selected narrow wavelength band. Each labelling phosphor may be detected by its spectrally separate colour. Thus binding of multiple labels may be quantitatively detected in each element of the array of assays.

Alternatively, as shown in Figure 18, the electron gun 236 may be of the type described in U.S. Patent 5,450,103 where the field electrode is capable of focusing the beam and directing the beam to any individual

element of a linear array of assays. Alternatively, the electron gun may be constructed like that of a TV tube where charged plates 238 and 240, as shown in Figure 18, focus and direct the beam to distinct locations on the two-dimensional grid 242. The electron gun includes the usual electron  
5 accelerating plates 244. The grid of test substrates 242 may in actual fact be an electrophoresis gel or blot of DNA clones which are being used to detect and determine DNA sequence. As shown in the enlarged view of the test substrate 246 a variety of different labels 248 are used which emit by virtue of their being doped with different metals, different colours of  
10 phosphorescence. Four distinct colours can designate the four distinct nucleotide bases to thereby provide an identification of the base at the end of each sequence.

### EXAMPLE 1

15

#### **Caging of Phosphor Microparticles with Glutaraldehyde Crosslinked Alkaline Phosphatase-Conjugated Avidin and Goat anti-human IgG**

Ten grams each of Y2O2S:Eu and ZnS:Cu:Al phosphors (Osram-  
20 Sylvania) were shaken in 750 mls of distilled water, then left to settle for 5 minutes. Two hundred mls of the lightly clouded suspensions were aspirated into 50 ml tubes and spun at 2800 RPM to separate the phosphors. The combined precipitates of each phosphor were resuspended in 5 mls of PBS pH7.2. Two aliquots of 0.5 ml each were treated with either Avidin-AP (100  
25 micrograms) or Caltag goat anti-human IgG-AP, allowing adhesive binding to take place at room temperature for 3 hours. The particles were resuspended by shaking at about 10 minute intervals during this time. Then 5 mls of PBS pH8.4 was added to wash the particles. The particles were then spun down and the supernatant discarded. PBS pH8.4 with 0.02% glutaraldehyde (5 mls  
30 PBS pH8.4 plus 100 microliters of a 1% glutaraldehyde-PBS-methanol stock solution) was added and the particles were tumbled on a rotary mixer for one hour. The particles were centrifuged out and the supernatant discarded. They were resuspended in 5 mls PBS pH8.4 and left rotating on the mixer

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overnight at room temperature. The next day they were washed twice more by centrifugation and resuspension in 2 mls blocking buffer (3 % BSA, 0.1M glycine in PBS pH8.4). The supernatant of the third wash was kept for assessment of residual enzyme activity. The particles were stored for a  
5 further 56 hours at room temperature and samples were taken for assessment of enzyme activity after vigorous washing and pipetting. The results are shown in Figure 19 and indicate efficient and stable crosslinked coating of the particles.

10

## **EXAMPLE 2**

### **Use of Goat anti-human IgG Coated Phosphor Particles in a Direct Detection Assay**

15 Goat anti-Human IgG coated ZnS:Cu:Al phosphor particles were prepared as described in Example 1 above. After further washing and resuspension in PBS pH7.2, 100 microliter aliquots of the particles were added to wells covalently coated with serially diluted human IgG. To maintain blocking, 100 ul of blocking buffer (1 % BSA) was added to each  
20 well. After incubation at room temperature for 48 hrs, the crosslinked particles were decanted, the wells were washed with distilled water and then PBS pH7.2. 100 microliters of the AP substrate was then added to each well. The results shown in Figure 20 indicates that the crosslinked goat anti-human IgG-AP bound to the wells in proportion to the concentration of human IgG  
25 bound.

## **EXAMPLE 3**

### **Direct Electron Beam Excitation of Microparticle Phosphors at Ambient Pressure**

30

The phosphors ZnS:Mn, Y<sub>2</sub>O<sub>2</sub>S:Eu, ZnS:Cu:Al, and ZnS:Ag were separately mixed into a thick paste with epoxy and cemented to the ends of

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stainless steel tubes (#19 hypodermic needle stock). Teflon heatshrink tubing was placed over the center portion of the shaft of the tube to electrically isolate the tube from the test fixture. The end of the tube was centered in front of one 100 micron aperture of a Delphax printhead array and the tube  
5 was connected through a nanoammeter to ground. The tip of the tube was positioned  $\frac{1}{4}$  to  $\frac{1}{2}$  millimeter away from the grid. Accelerating potentials of up to 1200 volts were applied to the grid aperture. Micron-sized plasmas of microsecond duration were generated beneath the grid by pulsed RF fields. Electrons created in the microscopic plasmas were accelerated through the  
10 grid toward the phosphor-coated tip of the tube. Visible light phosphorescence stimulated by direct electron bombardment could be seen by the naked eye, and was also observed with a macro-lens focused on the tip and aperture. All phosphors were excited directly at a distance of  $\frac{1}{4}$  mm. with a "turn-on" voltage of about 300 volts and current of about 0.3  
15 microamps. This experiment demonstrates the potential to use commercial high density array electron beam "printhead" to read out corresponding planar DNA array, or peptide array library assays on "chips".

Although preferred embodiments of the invention have been described herein in detail, it will be understood by those skilled in the art that variations  
20 may be made thereto without departing from the spirit of the invention or the scope of the appended claims.



**CLAIMS:**

1. A displacement competition assay for determining analyte concentration in a sample, said assay comprising the steps of:
  - 5 i) incubating a sample on a test surface having analyte recognition molecules and labelling entities associated with said recognition molecules, said labelling entities having particle reporters selected from the group consisting of dielectric particles, paramagnetic particles, phosphorescent particles, particles having a combination of at least two of dielectric,  
10 magnetic and phosphorescent properties, phages, phages incorporating reporter material and mixtures thereof, said labelling entities providing a base level signal;
  - ii) said sample analyte competing for at least one recognition site on said recognition molecule where binding of said sample analyte to said  
15 recognition molecule site displaces said labelling entities away from said test surface to define a test label signal; such displacement of said labelling entities being related to concentration of analyte in said sample;
  - iii) comparing said base label signal to said test label signal to determine concentration of sample analyte.
- 20 2. A competition assay of claim 1, wherein said recognition molecules are linked to said test surface, said labelling entities being linked to said recognition molecules, said sample analyte in complexing with said recognition molecule sites displacing said recognition molecule from said  
25 surface.
3. A competition assay of claim 1, wherein analyte entities have said labelling entities linked thereto, said analyte entities being complexed with said recognition molecule sites, said sample analyte competing with said  
30 labelled analyte entities for recognition molecule sites by displacing said labelled analyte entities from said recognition molecule sites and said sample analyte complexing with said recognition molecule sites; such displacement of said labelled analyte entities defining a test label signal.

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4. A competition assay of claim 3 wherein said recognition molecules are fixed to said test surface.
5. A competition assay of claim 2 wherein said recognition molecules are complexed with analyte entities fixed to said test surface.
6. A competition assay of claim 1, wherein said particles are dielectric, said label signal being measurable as a change in capacitance when said test surface forms part of a sensing capacitor.
- 10 7. A competition assay of claim 1, wherein said particles are phosphorescent particles, said label signal being a measure of emitted phosphorescent radiation when said test surface is stimulated to cause said particles to phosphoresce.
- 15 8. A competition assay of claim 1, wherein said particles are paramagnetic, said label signal being measurable as a change in inductance when said test surface is exposed to a magnetic field.
- 20 9. A competition assay of claim 1 wherein said particles are phages having dielectric properties, said label signal being a measurable charge in capacitance when said test surface forms part of a capacitor.
10. A competition assay of claim 1 wherein said particles are phages incorporating reporter material having at least one of a dielectric, magnetic or phosphorescent property.
- 25 11. A competition assay of claim 10 wherein said phages incorporate phosphorescent reporter materials, said label signal being detected as phosphorescent radiation when said test surface is stimulated to cause said particles to phosphoresce.
- 30

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12. A competition assay of claim 6, wherein said test surface is a plate of a sensing capacitor, said sample being introduced upon said plate.
13. A competition assay of claim 12 wherein said sensing capacitor is an  
5 opposing charged plate capacitor, said test surface being on one or both of said plates, said sample being introduced between said plates.
14. A competition assay of claim 13 wherein said sample flows between said plates due to capillary attraction between closely spaced plates of said  
10 capacitor.
15. A competition assay of claim 7 wherein said phosphorescent particles are excited by electromagnetic radiation at a wavelength tuned to phosphorescent particles.  
15
16. A competition assay of claim 1, wherein said reporter particles are individually of nanometre size in the range of 1 to 1000 nm.
17. A competition assay of claim 7 wherein said reporter particles are  
20 individually of nanometre size in the range of 0.5 to 20 nm.
18. A competition assay of claim 1 wherein said reporter particles are individually of micron size in the range of 1 to 1000  $\mu\text{m}$ .
- 25 19. A competition assay of claim 9, wherein analyte entities have said labelling entities linked thereto, said analyte entities being complexed with said recognition molecule sites, said sample analyte competing with said labelled analyte entities for recognition molecule sites by displacing said labelled analyte entities from said recognition molecule sites and said sample  
30 analyte complexing with said recognition molecule sites; such displacement of said labelled analyte entities defining a test label signal, said phages expressing on their surface molecules which form binding sites which

recognize a portion of said analyte entity different from a portion of said analyte entity recognized by said fixed recognition molecules.

20. A competition assay of claim 10, wherein analyte entities have said  
5 labelling entities linked thereto, said analyte entities being complexed with  
said recognition molecule sites, said sample analyte competing with said  
labelled analyte entities for recognition molecule sites by displacing said  
labelled analyte entities from said recognition molecule sites and said sample  
analyte complexing with said recognition molecule sites; such displacement of  
10 said labelled analyte entities defining a test label signal, said phages  
expressing on their surface molecules which form binding sites which  
recognize a portion of said analyte entity different from a portion of said  
analyte entity recognized by said fixed recognition molecules.

15 21. A test component of a diagnostic apparatus for conducting an assay for  
determining analyte concentration in a sample, said test component  
comprising a test surface having recognition molecules for said analyte and  
labelling entities associated with said recognition molecules, said labelling  
entities having particle reporters selected from the group consisting of  
20 dielectric particles, magnetic particles, phosphorescent particles, particles  
having a combination of at least two of dielectric, magnetic and  
phosphorescent properties, phages and phages incorporating reporter material  
and mixtures thereof.

25 22. A test component of claim 21 wherein said recognition molecules are  
linked to said test surface, said labelling entities being linked to said  
recognition molecules.

23. A test component of claim 21 wherein analyte entities are provided and  
30 have said labelling entities linked thereto, said analyte entities being  
complexed with said recognition molecule sites.

24. A test component of claim 23 wherein said recognition molecules are fixed to said test surface.
25. A test component of claim 22 wherein said recognition molecules are  
5 complexed with analyte entities fixed to said test surface.
26. A test component of claim 21, wherein said test surface forms part of said sensing capacitor, said reporter particles being dielectrical particles, dielectric phages or phages expressing dielectric molecules.
- 10 27. A test component of claim 21, wherein said test surface is susceptible to electromagnetic radiation emitted by a diagnostic apparatus which excites selected phosphorescent particles to phosphoresce.
- 15 28. A test component of claim 21, said test surface is susceptible to a magnetic field emitted by a diagnostic apparatus and said reporter particles are paramagnetic.
- 20 29. A test component of claim 21, wherein said particles are individually of nanometre size in the range of 1 to 1000 nm.
30. A test component of claim 21, wherein said particles are individually of nanometre size in the range of 0.5 to 20 nm.
- 25 31. A test component of claim 21, wherein said particles are individually micron size in the range of 1 to 1000  $\mu\text{m}$ .
- 30 32. A test component of claim 26, wherein said sensing capacitor is a parallel plate capacitor provided as part of an electronic semi-conductor micro-chip, said micro-chip having closely spaced apart component surfaces to provide a capillary through which a test sample containing suspected analyte may pass.



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33. A test component of claim 21, wherein:

i) said test surface is associated with a first conductor of a sensing capacitor, said selected particles being dielectric;

5 ii) a circuit means, electrically coupled to said sensing capacitor responsive to changes in capacitance, said change in capacitance being due to displacement of said dielectric particle labelling entities by sample analyte in a test sample introduced to said sensing capacitor.

34. In an assay for detecting presence of a suspected analyte in a test  
10 sample by use of an analyte recognition molecule, the use of phage particles as reporter labels in identifying presence of sample analyte.

35. In an assay of claim 34, said phage particles are dielectric and are used in an assay format selected from the group consisting of competition assay,  
15 sandwich immunoassay, direct immunoassay, indirect immunoassay and nucleotide hybridization assays.

36. In an assay of claim 34, said phage particles express on their surface molecules which define recognition sites for said analyte.

20

37. In an assay of claim 34, said phage particles express on their surface molecules which define recognition sites for analyte entities used in a competition assay.

25 38. In an assay of claim 37, said phage particles are dielectric, said assay being a competition assay using a test surface comprising analyte recognition molecules bound to said test surface, said recognition molecules being complexed with analyte entities wherein said recognition sites of said phage particles recognize said analyte entities to present a test surface having a  
30 measurable dielectric constant.

39. In an assay for detecting presence of a suspected analyte in a test sample by use of an analyte recognition molecule, the use of solid phase semi-conductor polymer phosphors as reporter labels in identifying presence of sample analyte complexed with said recognition molecules.

5

40. In an assay of claim 39, said phosphors are used in an assay format selected from the groups consisting of competition assay, sandwich immunoassay, direct immunoassay, indirect immunoassay, nucleotide hybridization assays and nucleotide sequencing assays.

10

41. In an assay of claim 39, said phosphors being linked to recognition molecules capable of complexing with either analyte or analyte entities.

15 42. In an assay of claim 39, said phosphors being linked to analyte entities which in turn are complexed with said recognition molecule to adapt said assay to a competition assay.

20 43. In an assay of claim 39, said phosphors being stimulated to phosphoresce by a source of electromagnetic radiation selected from light or electrons, the latter being generated by field emission, electron gun or an electrical potential applied across or through the test surface.

25 44. In an assay of claim 39, 40, 41, 42 or 43, said semi-conductor polymer phosphor being selected from one or more of the following:

i) a polymer selected from the group consisting of poly(vinylcarbazole), poly(paraphenylene), parahexaphenyl, poly(perylen-co-diethynylbenzene), poly(aniline),  
30 poly(phenylenevinylene), poly(thienylene), poly(thienylenevinylene), poly(phenylene sulfide), poly (9,9-dihexylfluorene), and poly (methylphenylsilane); and

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ii) a polymer selected from group i) doped with a member selected from the group consisting of phthalocyanines, naphthalocyanines, metalloporphyrins, Thiapyrylium, Perylenes, Quinacridones, Squarilium 9,10-bis(styryl)anthracene, electron transporters hole transporters, polycyclic  
5 organic fluorescent dopants, inorganic nanocrystalline semiconductors, lanthanide cryptates or lanthanide chelates.

45. In an assay of claim 44, wherein said electron transporter is selected from the group consisting of poly(oxadiazols), fluorenylidene malonitrile,  
10 diphenoquinones, fullerenes, and aluminum quintolates.

46. In an assay of claim 44, wherein said hole transporter is a poly(triarylamine).

15 47. In an assay of claim 44, wherein said polycyclic organic fluorescent dopant is selected from the group consisting of N,N'-bis((2,5-tert-butylphenyl)-3,4,9,10-perylenedicarboximide, N,N'-bis((2,6-dimethylphenyl)-3,4,9,10-perylenetetracarboxylic diimide, N,N'-bis((3-aminophenyl)-3,4,9,10-perylenetetracarboxylic diimide,  
20 fluorenones, and dibenzothiophenes.

48. In an assay of claim 44, wherein said inorganic nanocrystalline semiconductor is selected from the group consisting of ZnS:Mn, CdS:Mn and ZnS:Tb.

25

49. A phage reporter particle comprising a phage incorporating reporter material having a property selected from the group consisting of dielectric properties, magnetic properties, phosphorescent properties and mixtures thereof.

30

50. A phage reporter particle of claim 44, wherein said selected particle is of nanometre size.

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51. A phage reporter particle of claim 44 wherein said selected particle is of micron size.

52. An apparatus for detecting the presence of a suspected analyte involving the use of phosphorescent labels, said apparatus comprising:

- i) a test substrate transparent to phosphorescent radiation from such phosphorescent label associated with said test substrate;
- ii) an electron beam generating device for emitting charged particles in the form of a defined beam, an electron generator having two electrodes separated by a dielectric material, a field electrode in front of said electron generator for focusing moving electrons into a beam and directing such beam;
- iii) said test substrate being spaced from said field electrode in air less than 1 mm,
- iv) phosphorescent detector located behind said test substrate to detect any phosphorescence emitted by such phosphorescent labels excited by such focused electron beam.

20

53. An apparatus of claim 52 wherein a two-dimensional array of a plurality of said electron beam generating devices are provided, each device being focused on a corresponding test substrate which is fixed in a corresponding two-dimensional array of test substrates, said apparatus being thereby adapted for multiple assays.

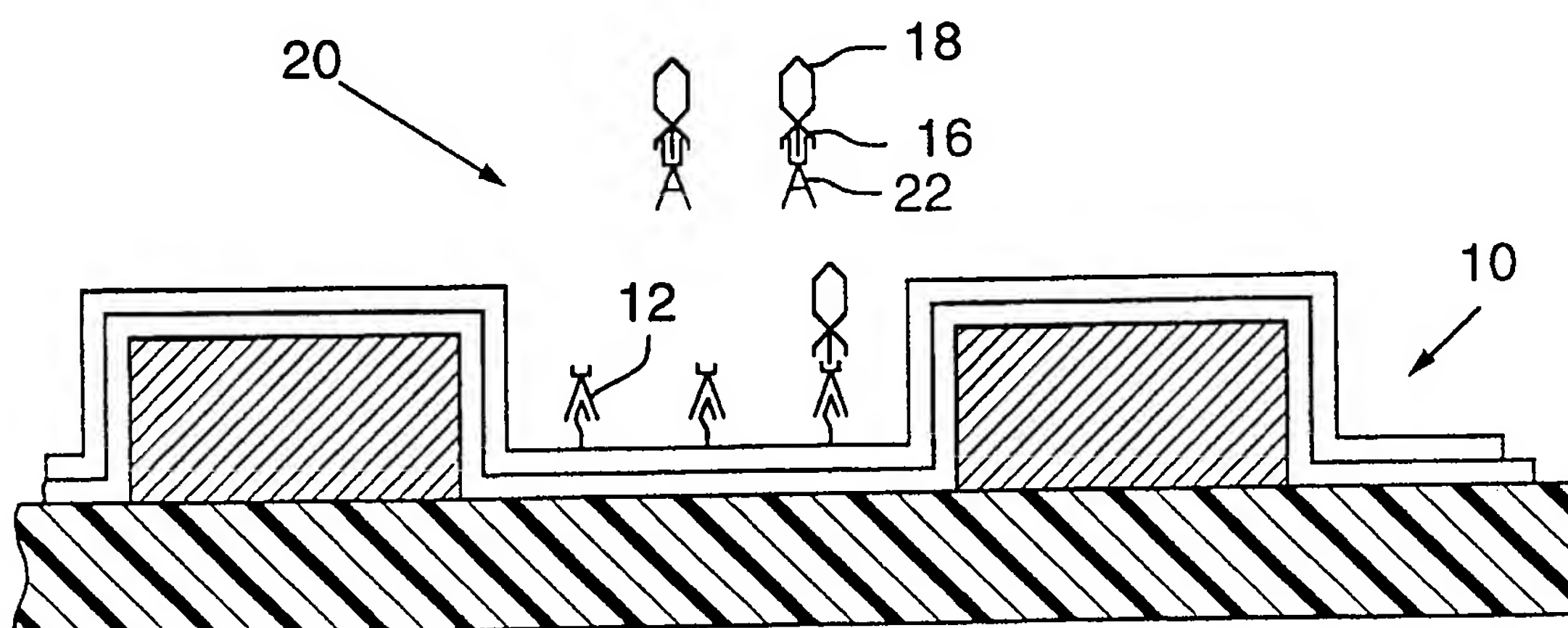
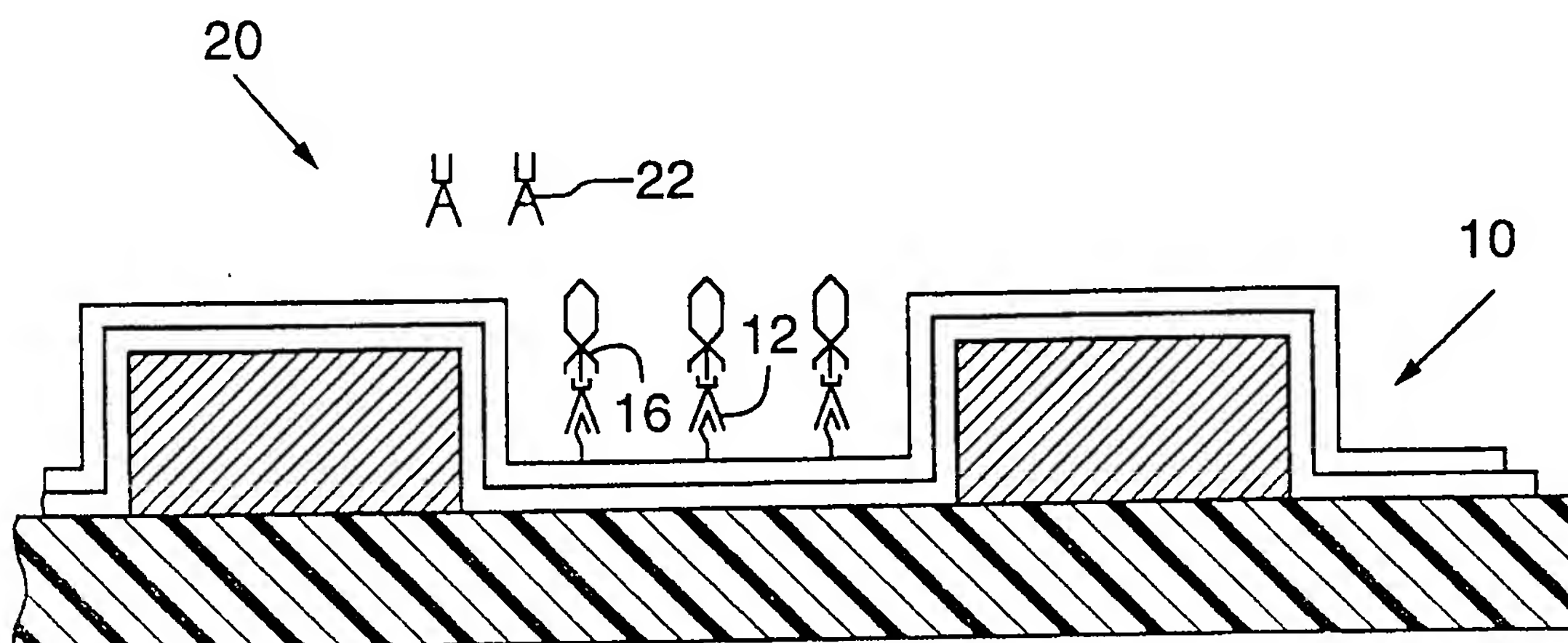
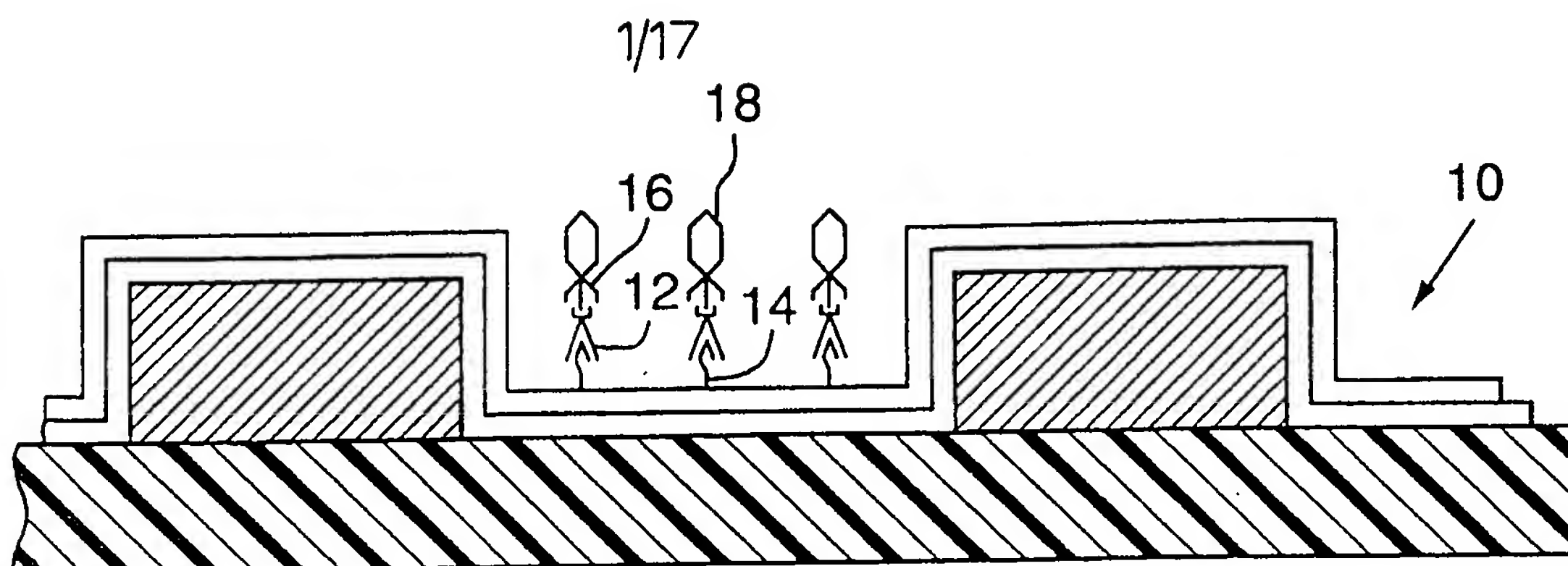
25

54. An apparatus of claim 52 wherein said field electrode is adapted to focus an electron beam on at least two spaced apart test substrate which are arranged in a two-dimensional array.

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55. An apparatus of claim 53 or 54 adapted for use in nucleic acid sequencing or hybridization assays, said two-dimensional array of test substrates being an electrophoresis gel, a membrane, or any solid phase or surface carrying an ordered two dimensional array of nucleic acid sequences, genes, or cloned gene fragments.
- 5





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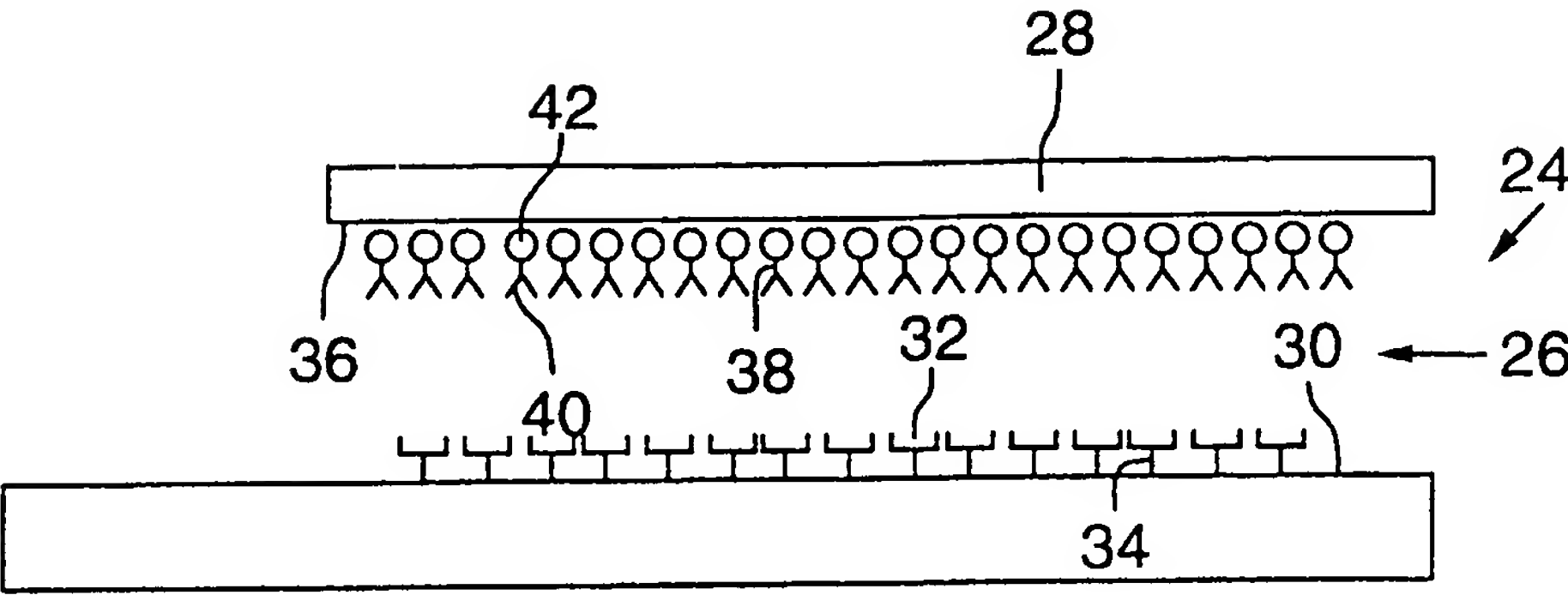


FIG. 2A

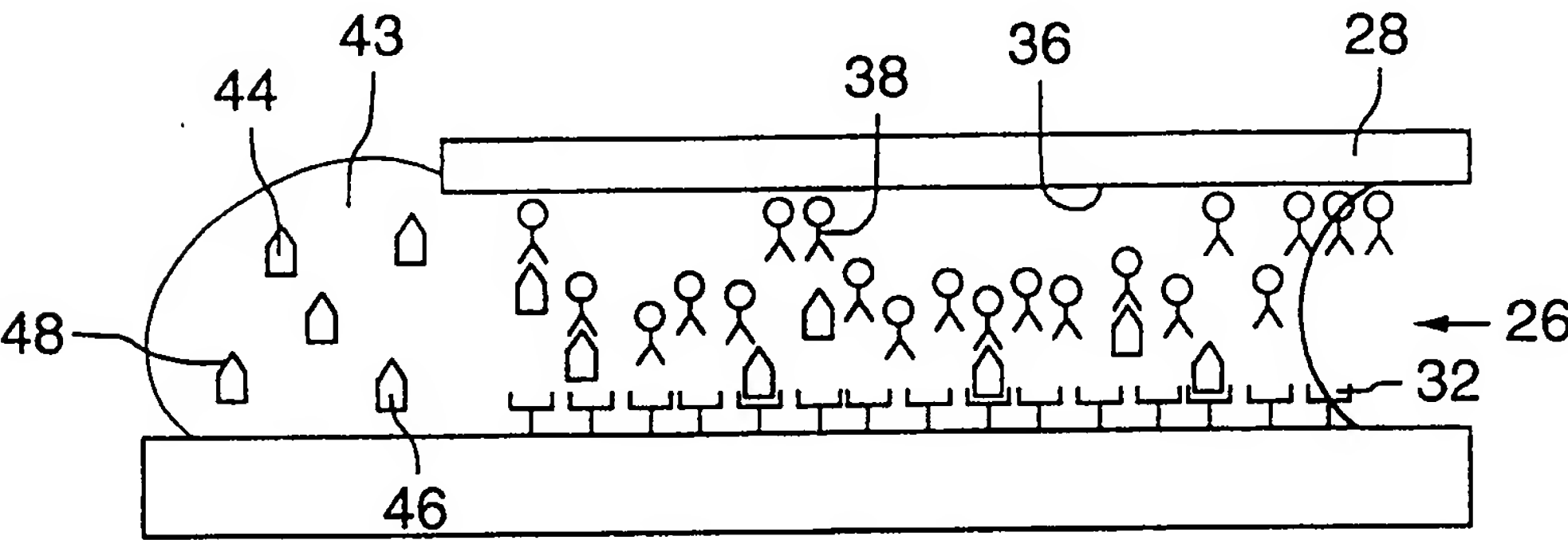


FIG. 2B

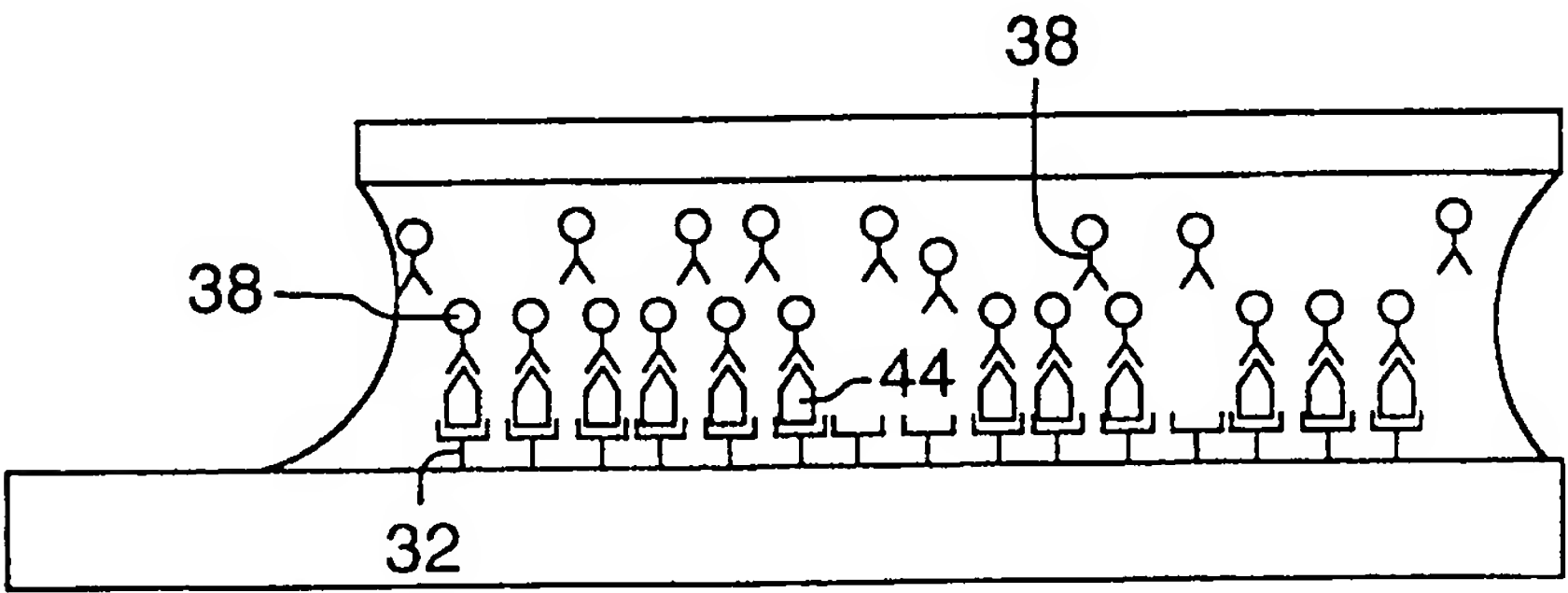


FIG. 2C

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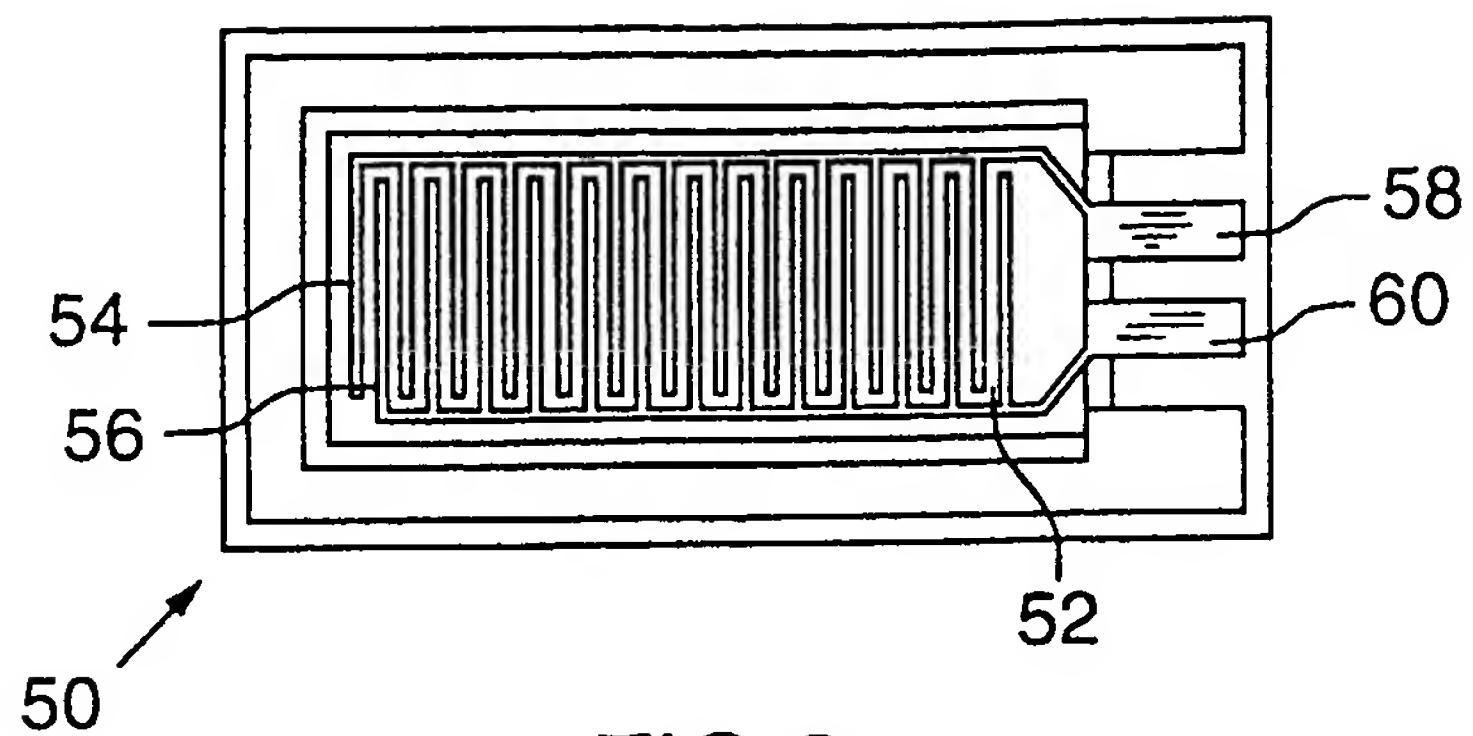


FIG. 3

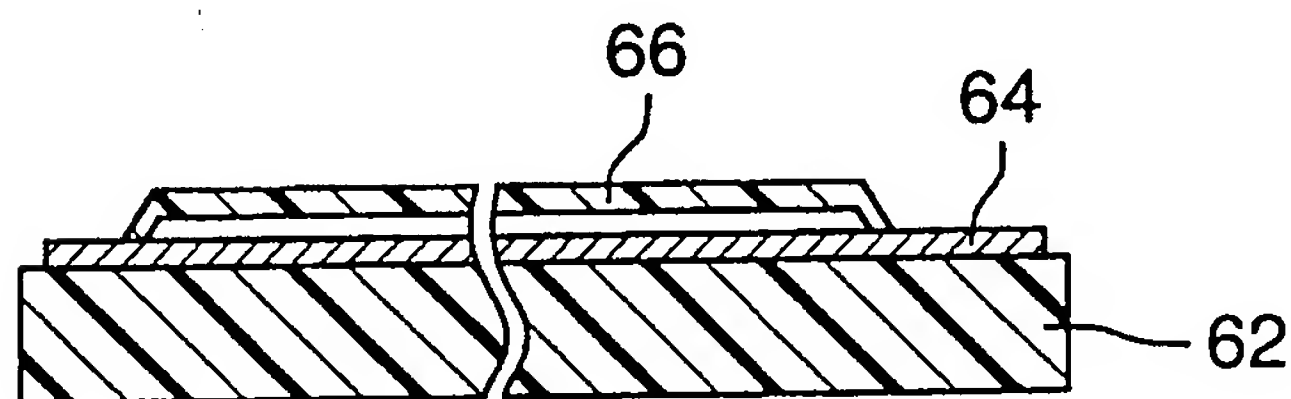


FIG. 4

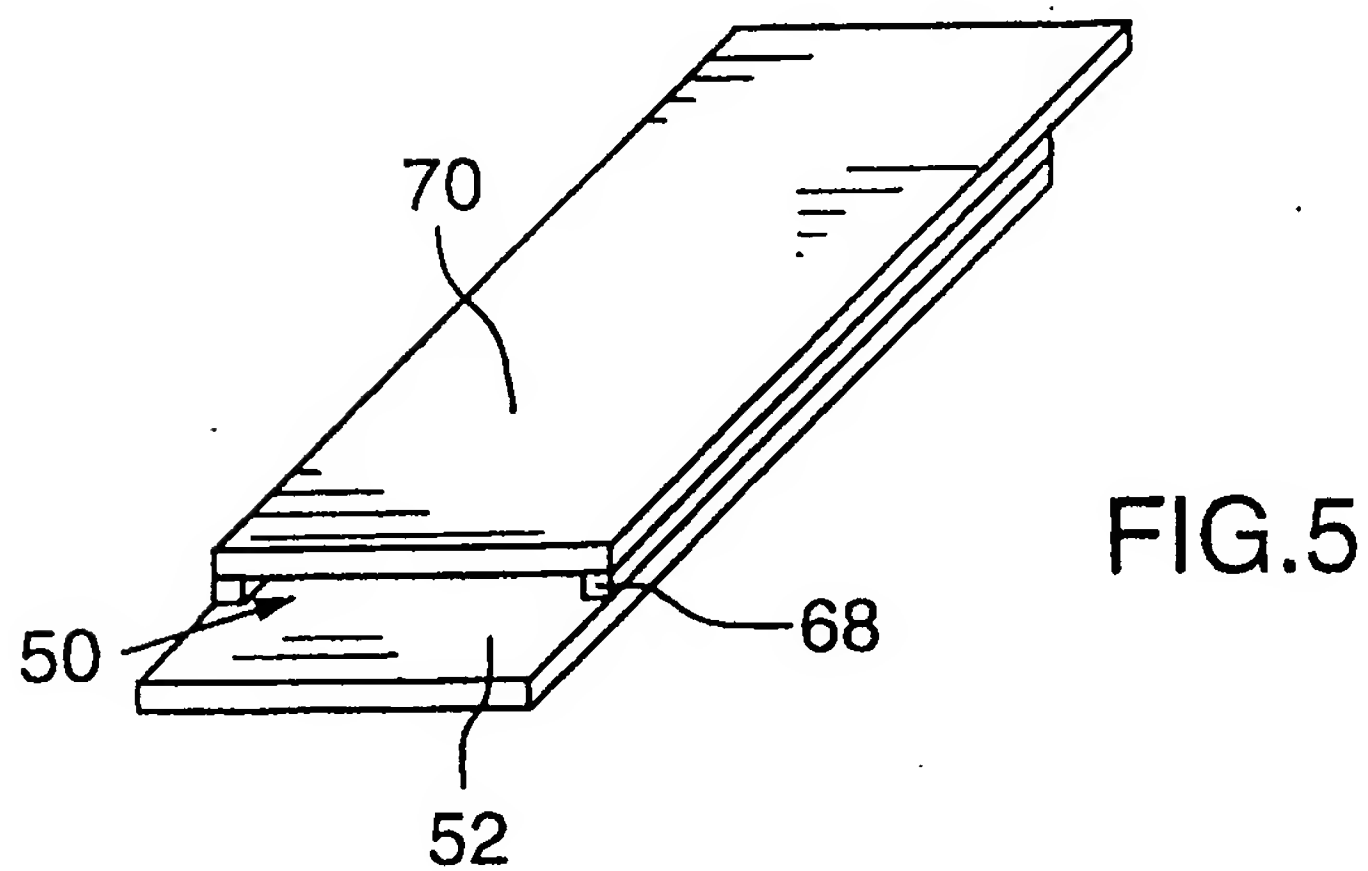


FIG. 5

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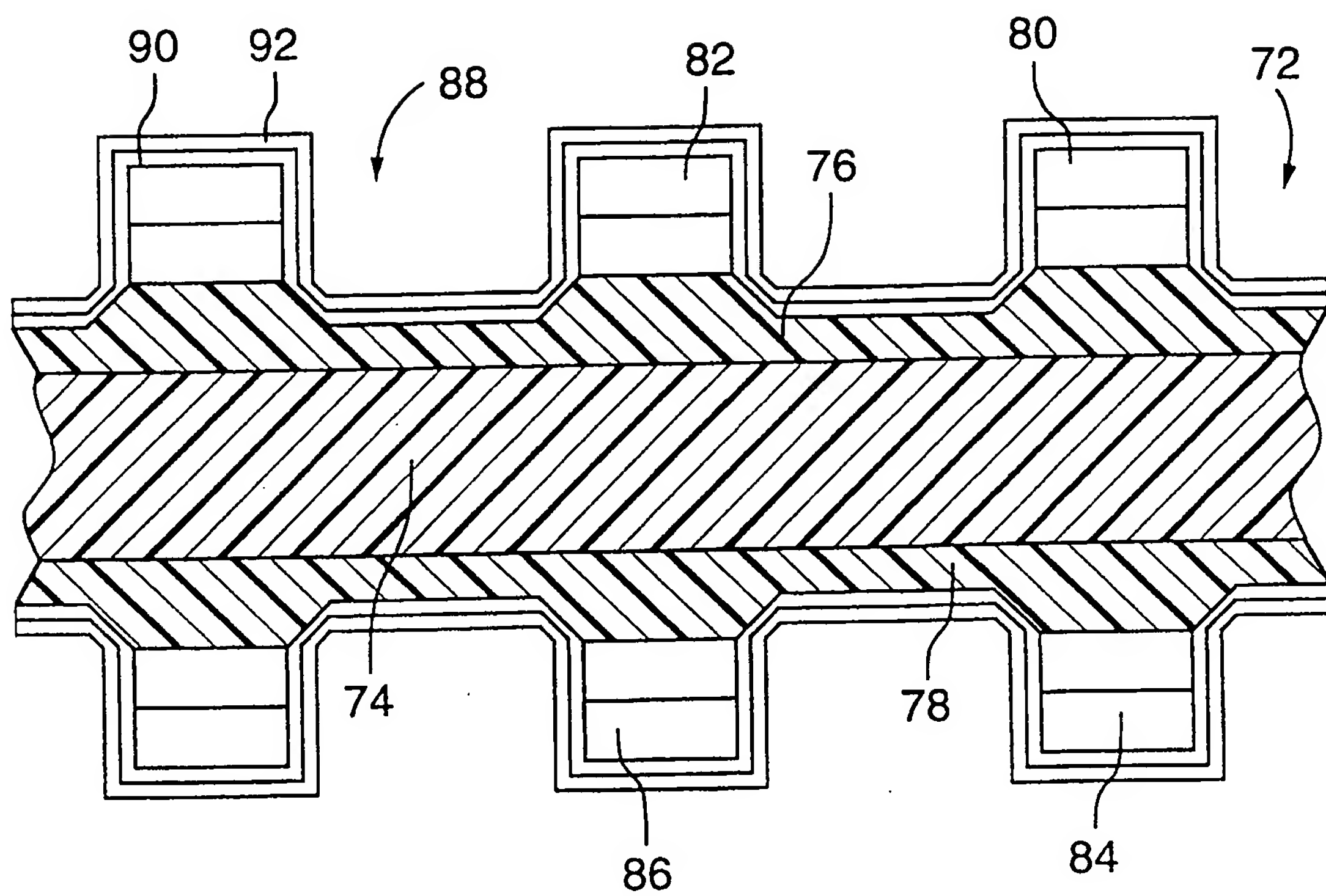
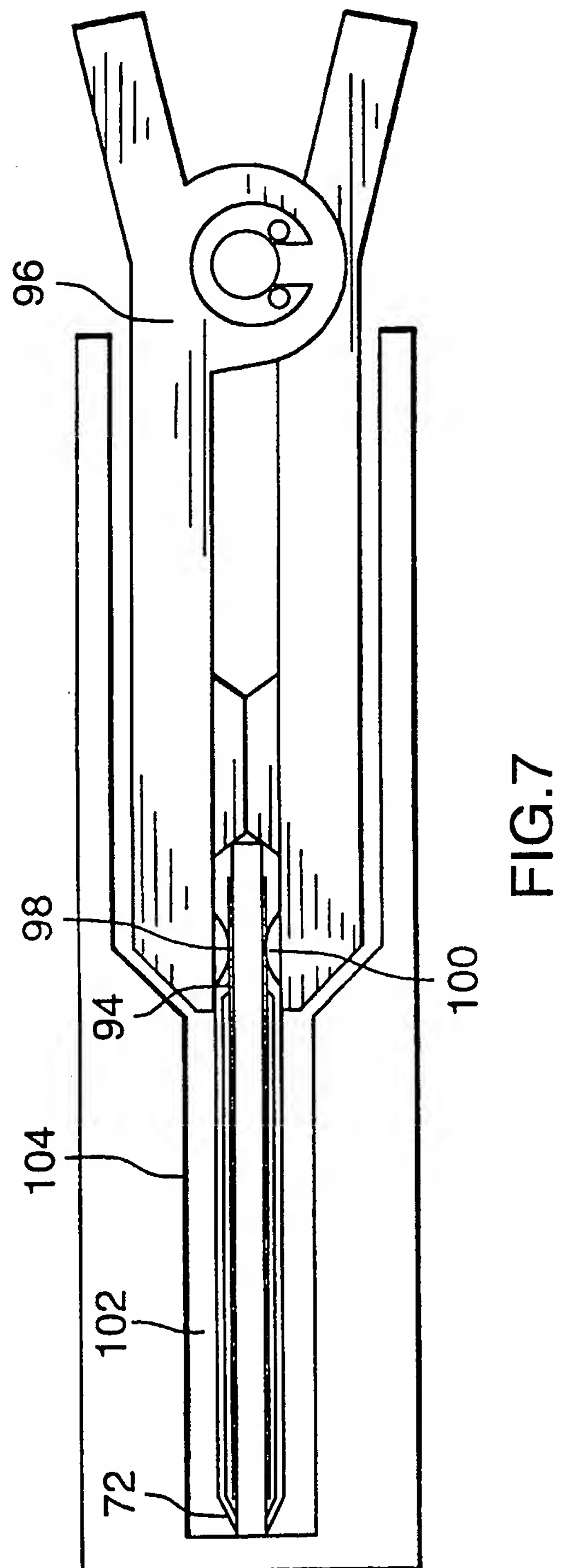


FIG.6

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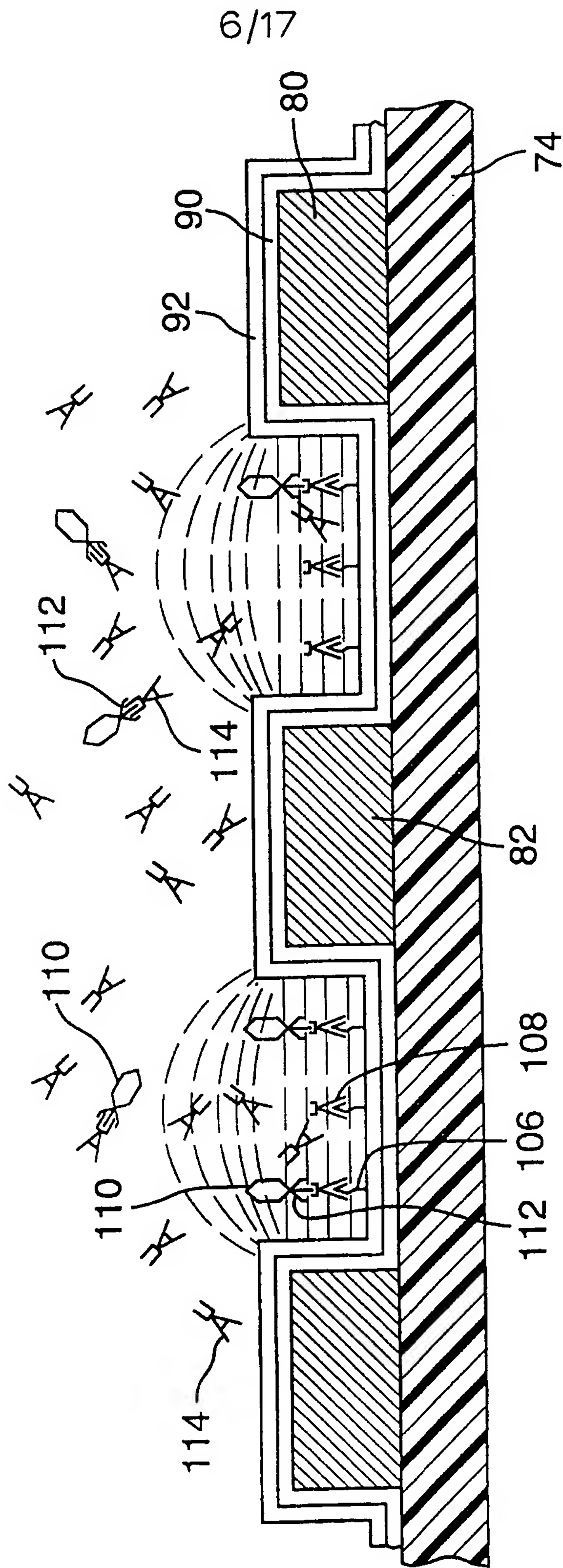


FIG.8

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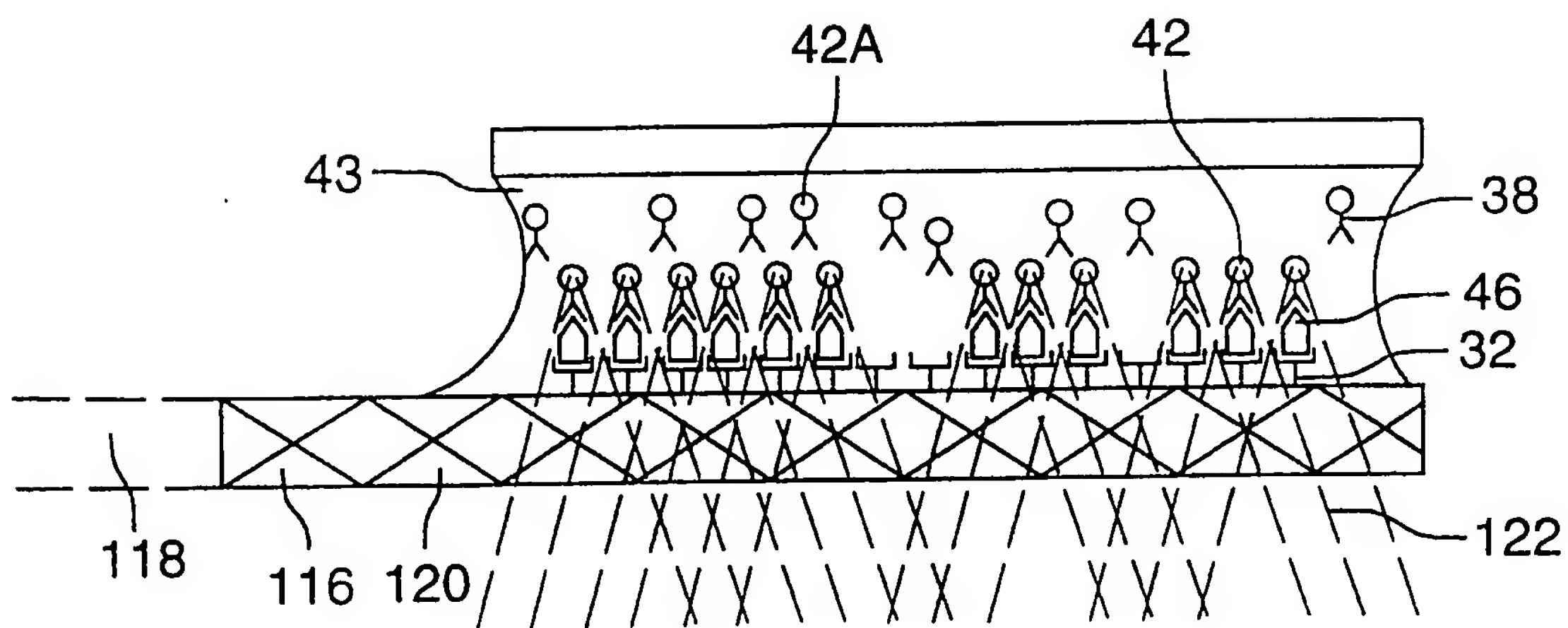


FIG.9

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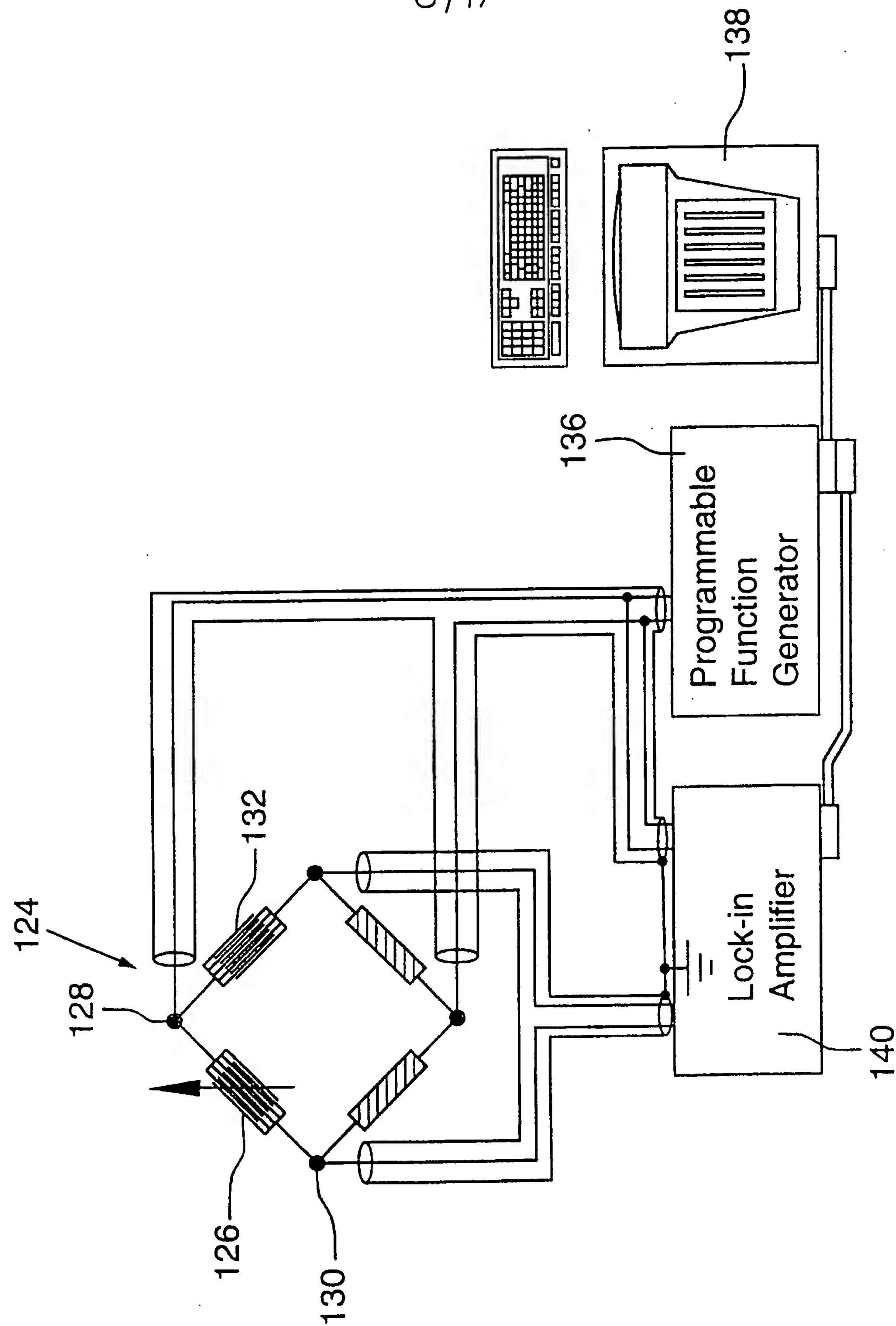
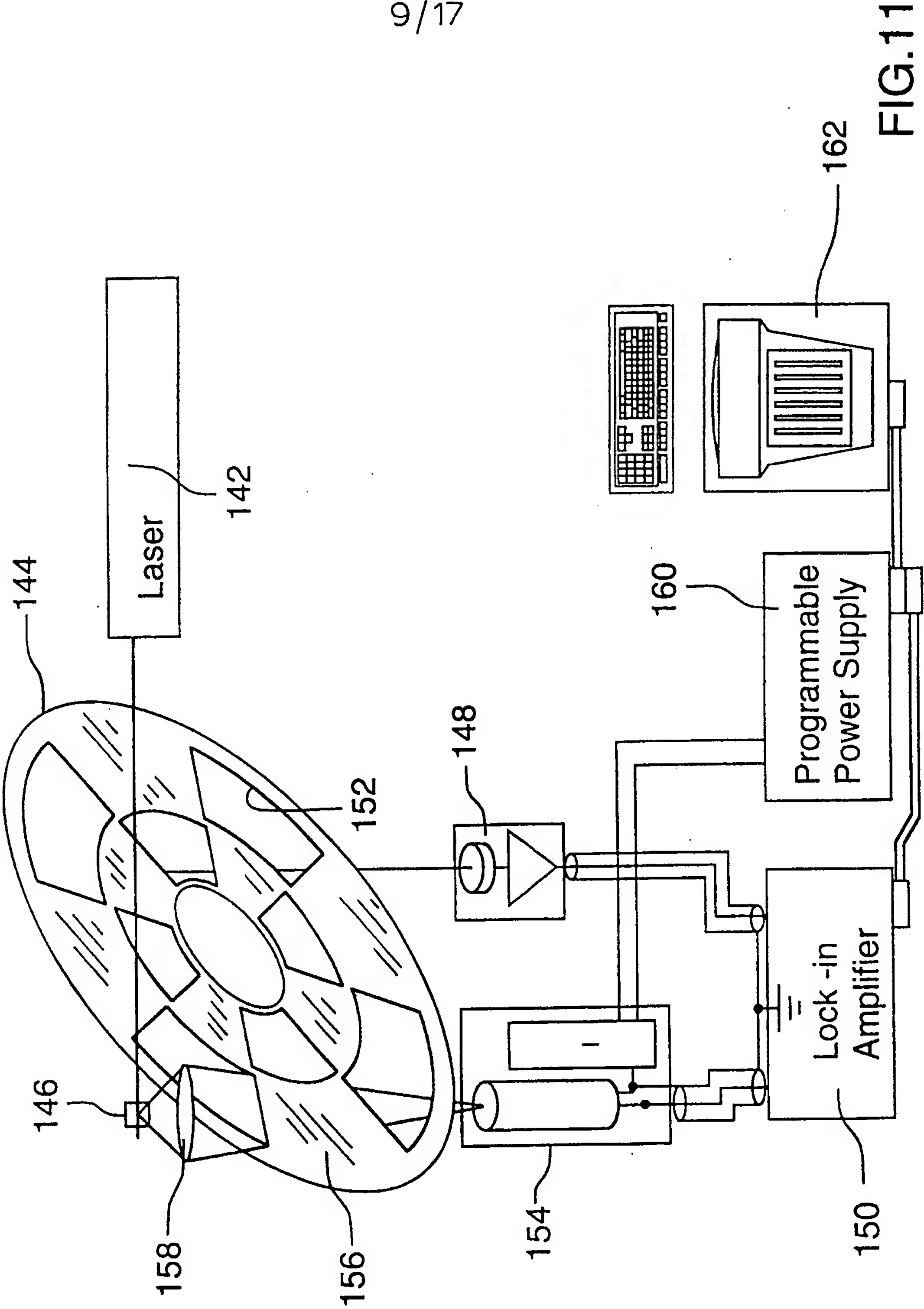


FIG.10



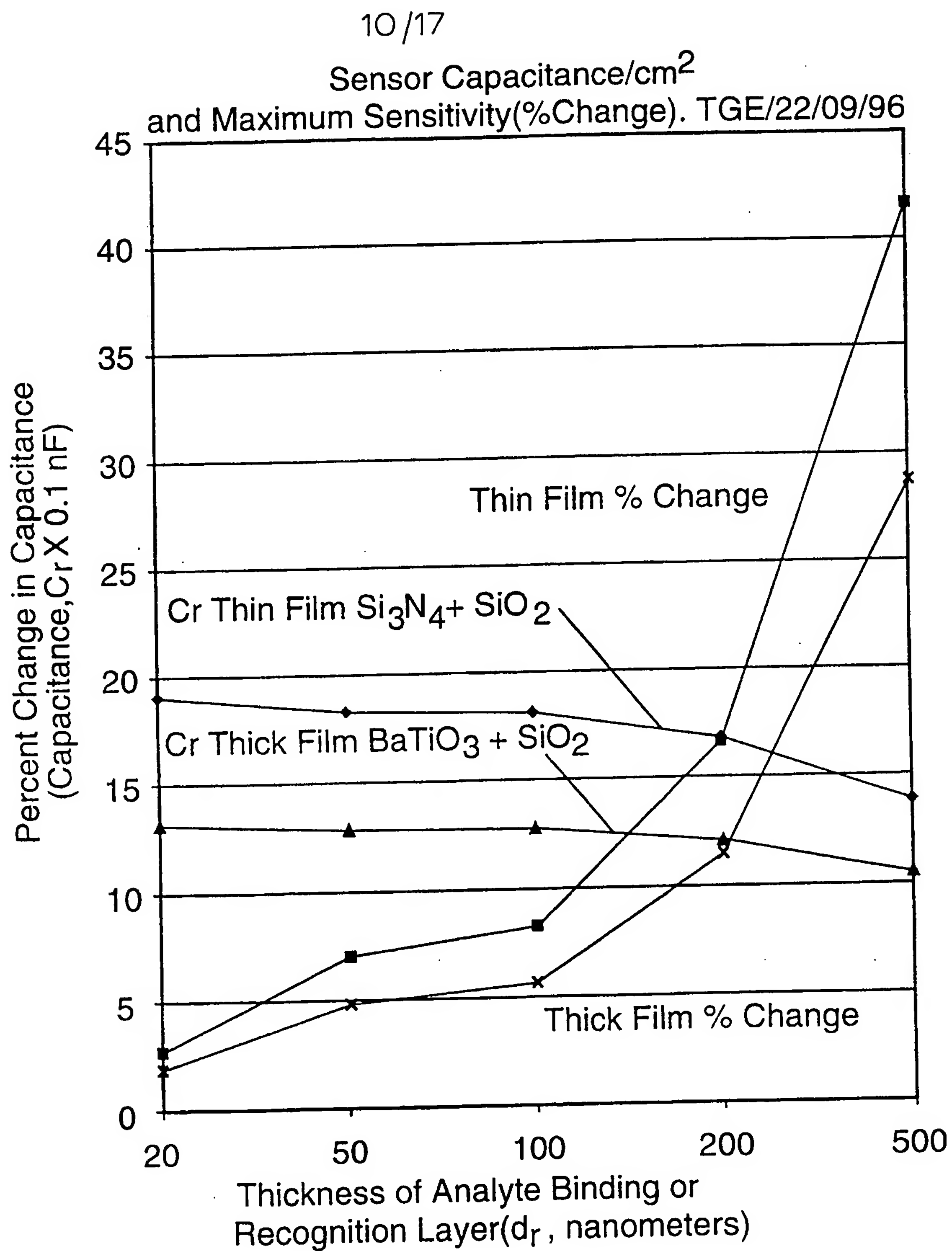


FIG.12



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Sensor Capacitance/cm<sup>2</sup> and Maximum  
Sensitivity (% Change)- Impact of No Glass  
Passivation Layer. TGE/22/09/96

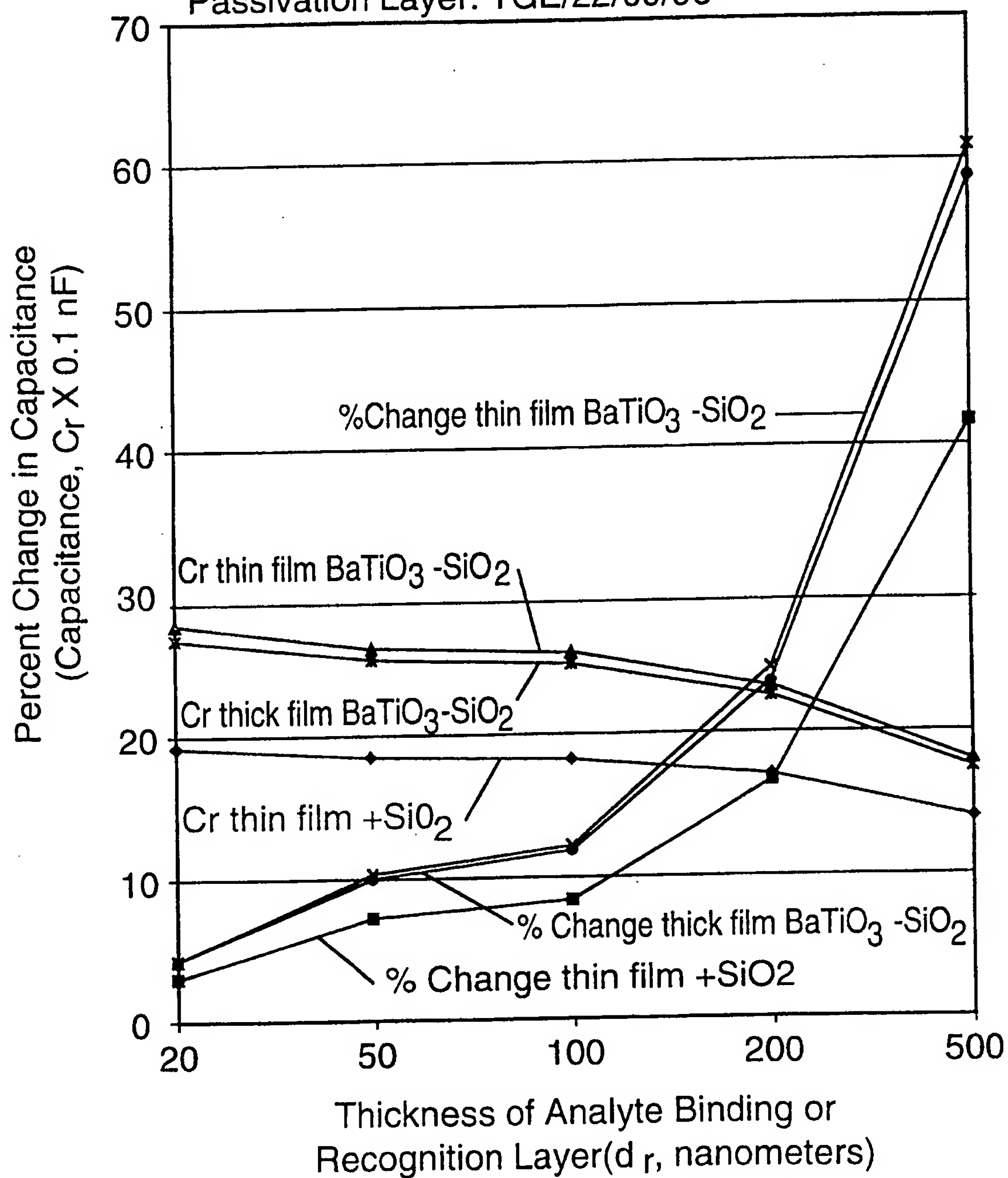


FIG.13

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Capacitance/cm<sup>2</sup> and Maximum Sensitivity (%  
Change) for Planar Interdigitated Thin Film Structure  
With 1.6 Micron High Faces and 5 Micron Wide Gaps-  
With and Without Glass Passivation. (TGE/23/09/96)

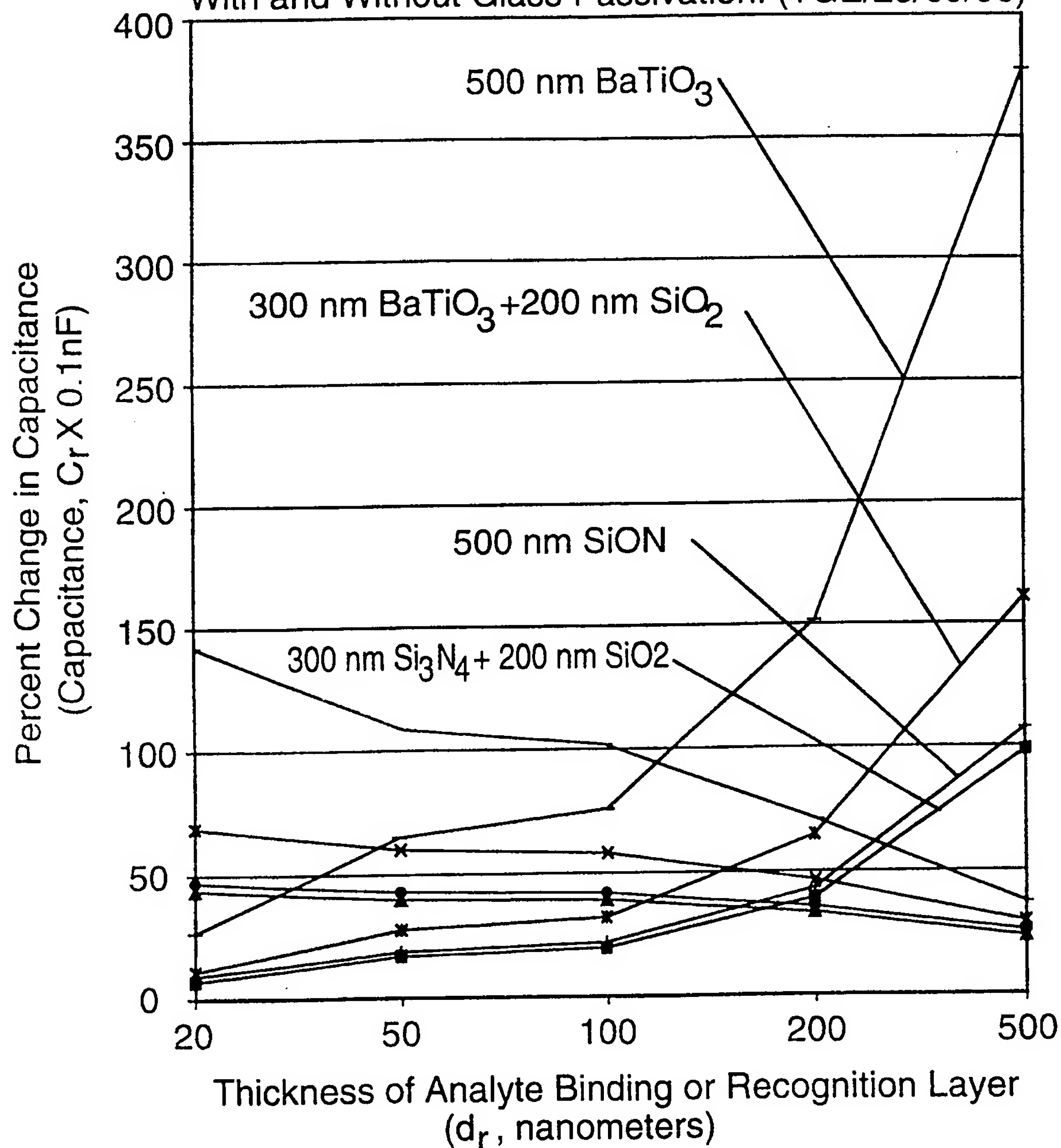


FIG.14

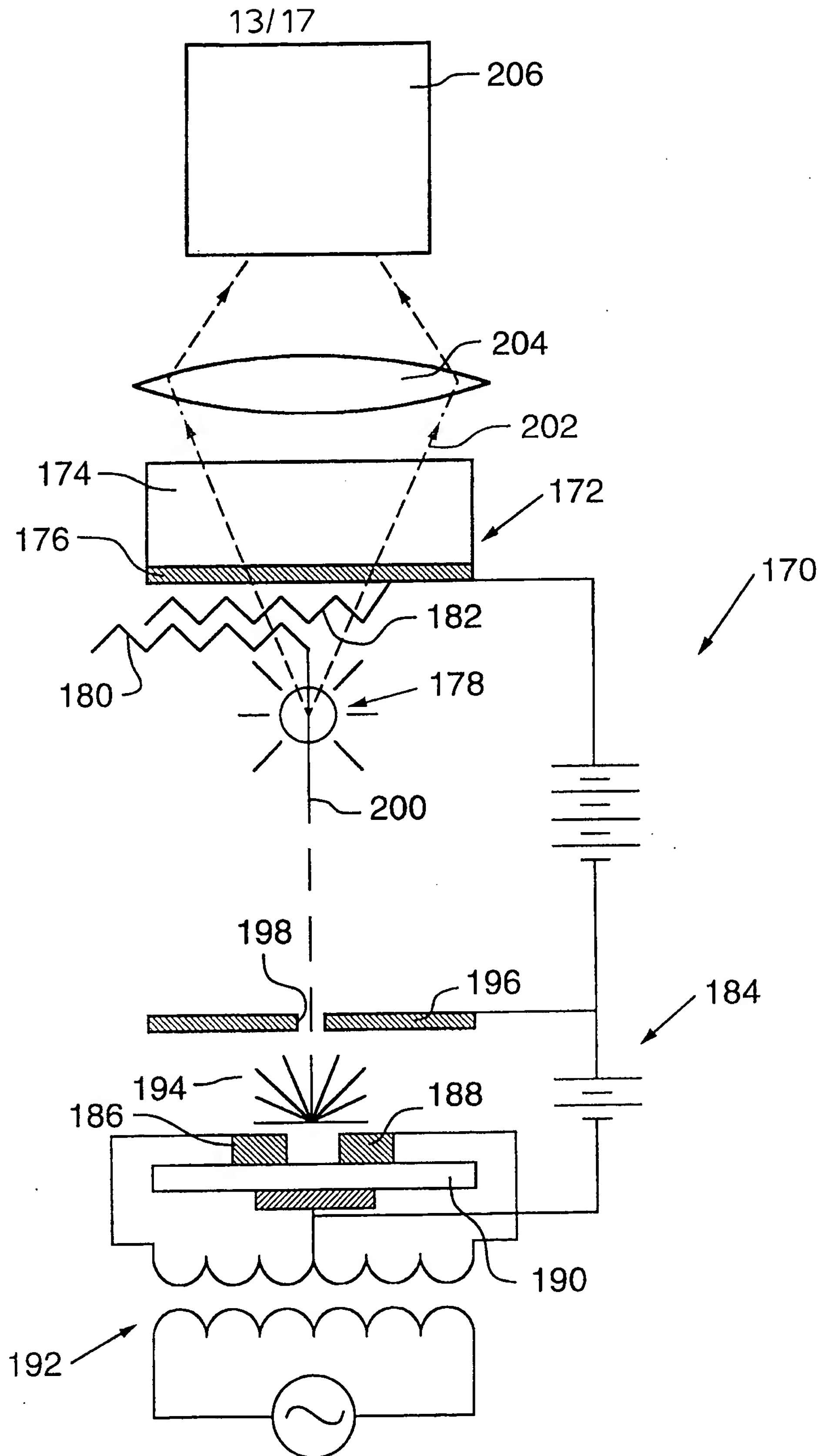
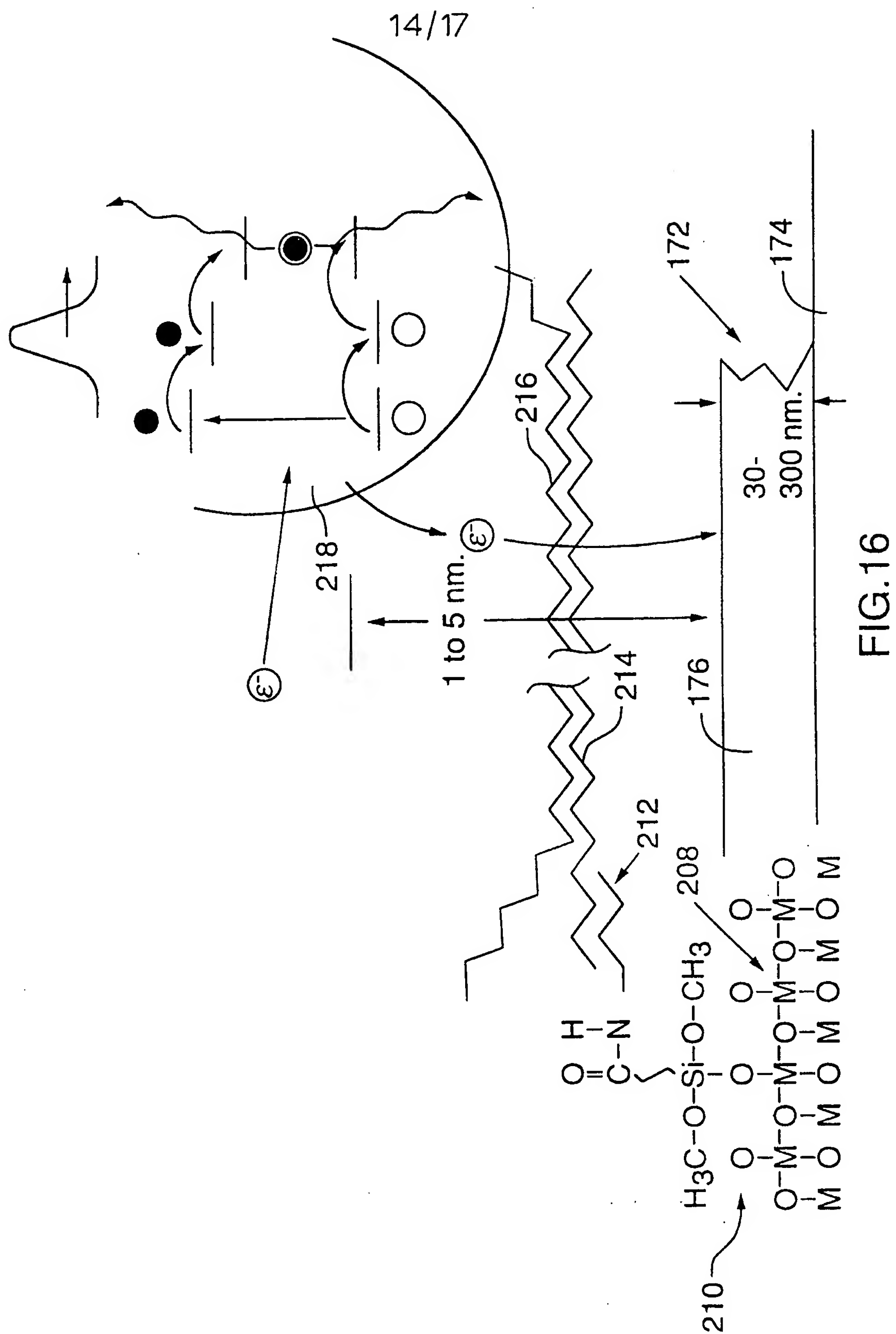


FIG. 15

**SUBSTITUTE SHEET (RULE 26)**



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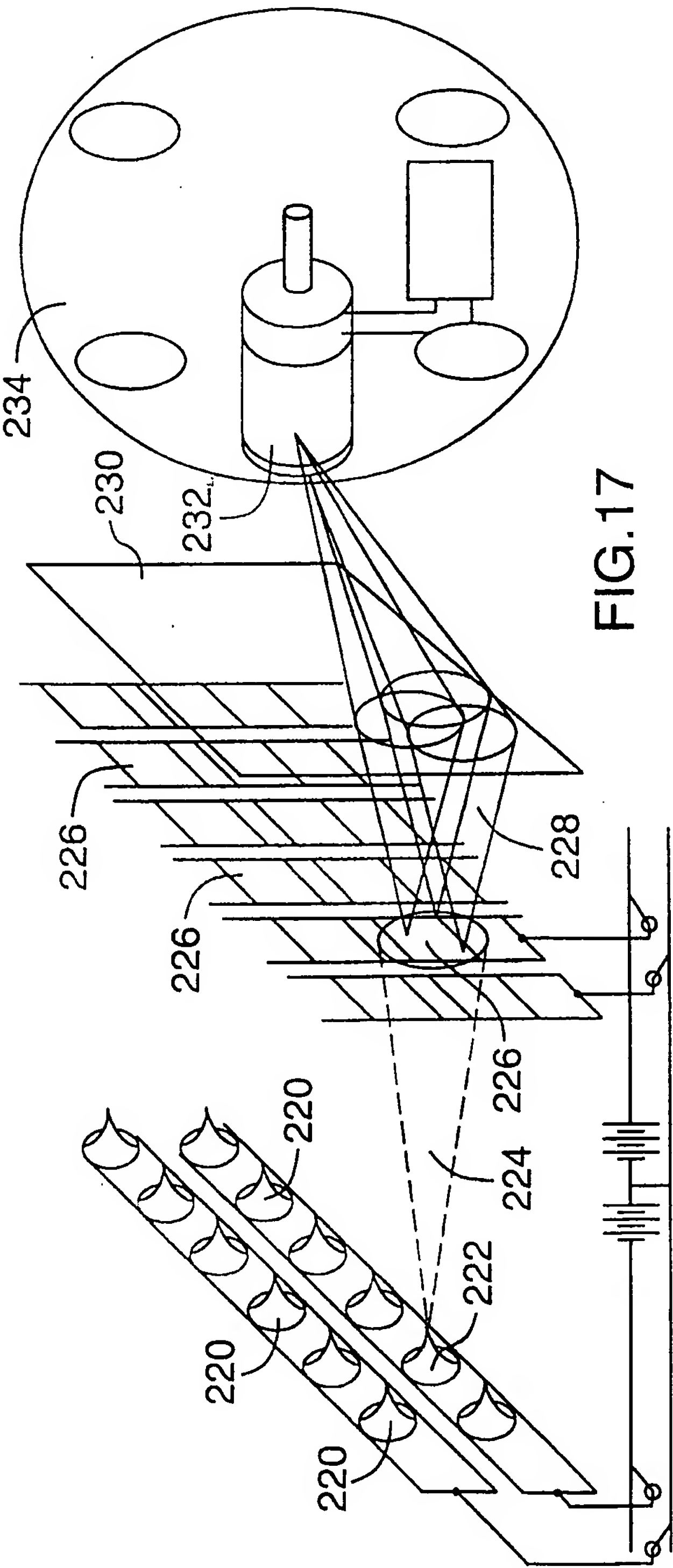


FIG. 17

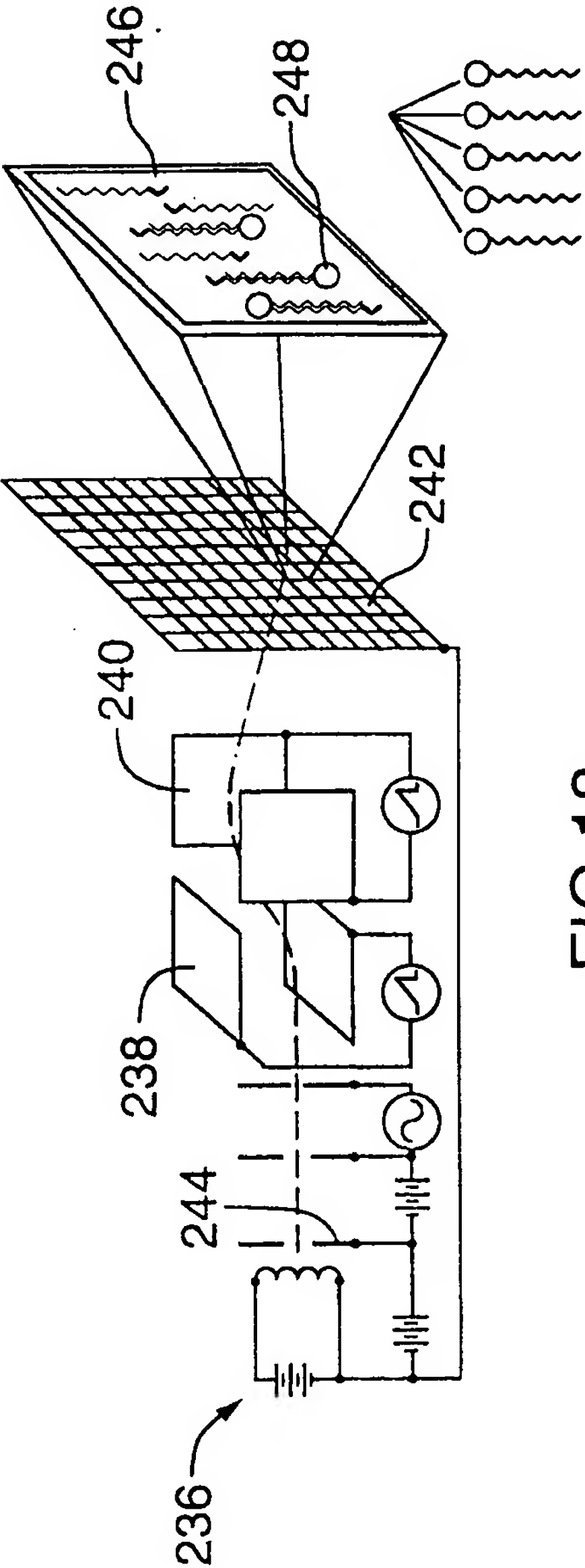


FIG. 18



Crosslinking of Avidin-AP and Goat-Anti-Human-AP to ZnS:Cu:Al and Y2O2S:Eu Phosphor Particles.  
(97/09/04/TGE)

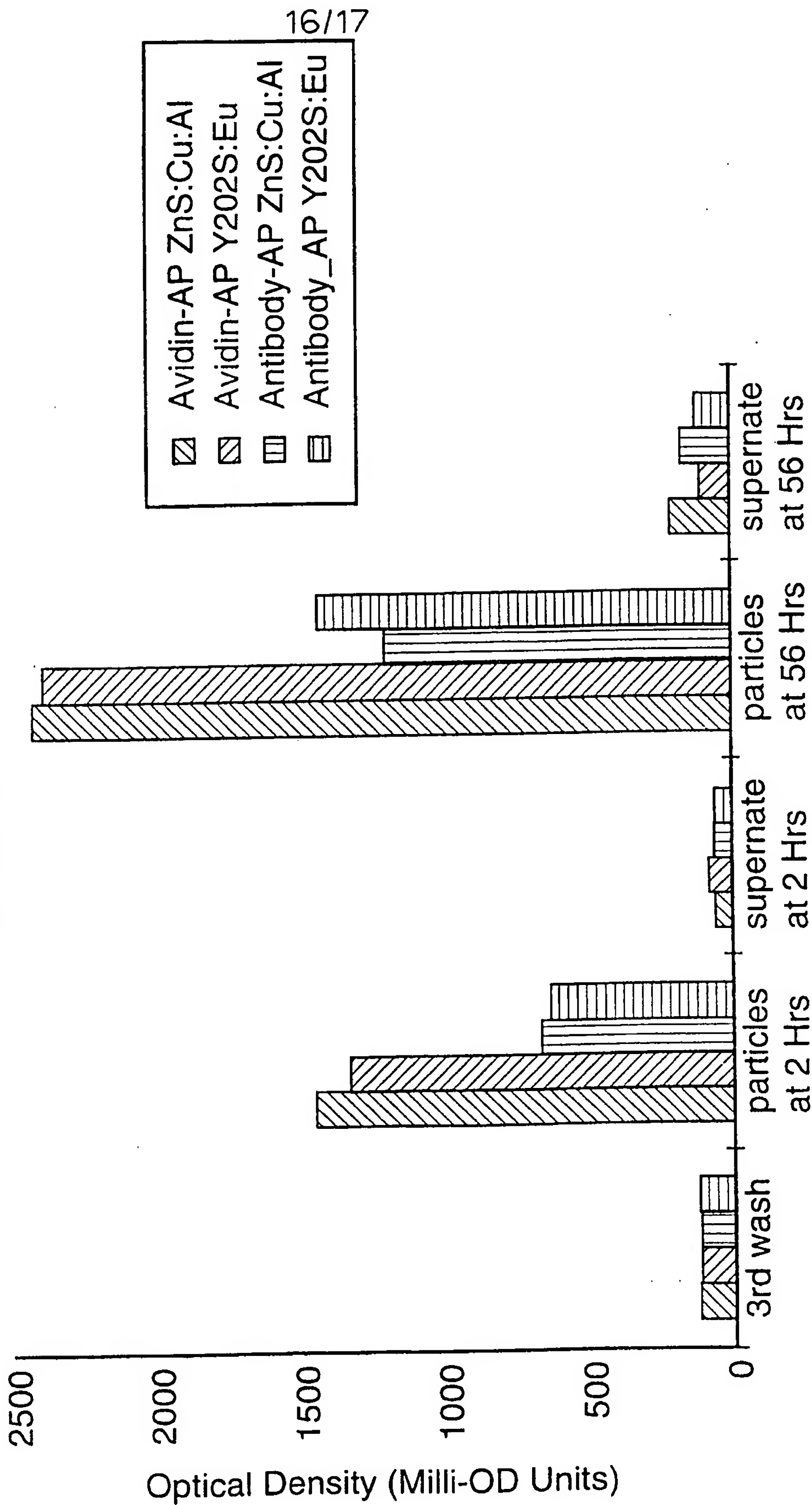


FIG.19

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Amino-PS Wells Covalently Coated With higG.Crosslinked  
Goat-Anti-Human IgG-Ap Phosphors.(97/09/04/TGE)

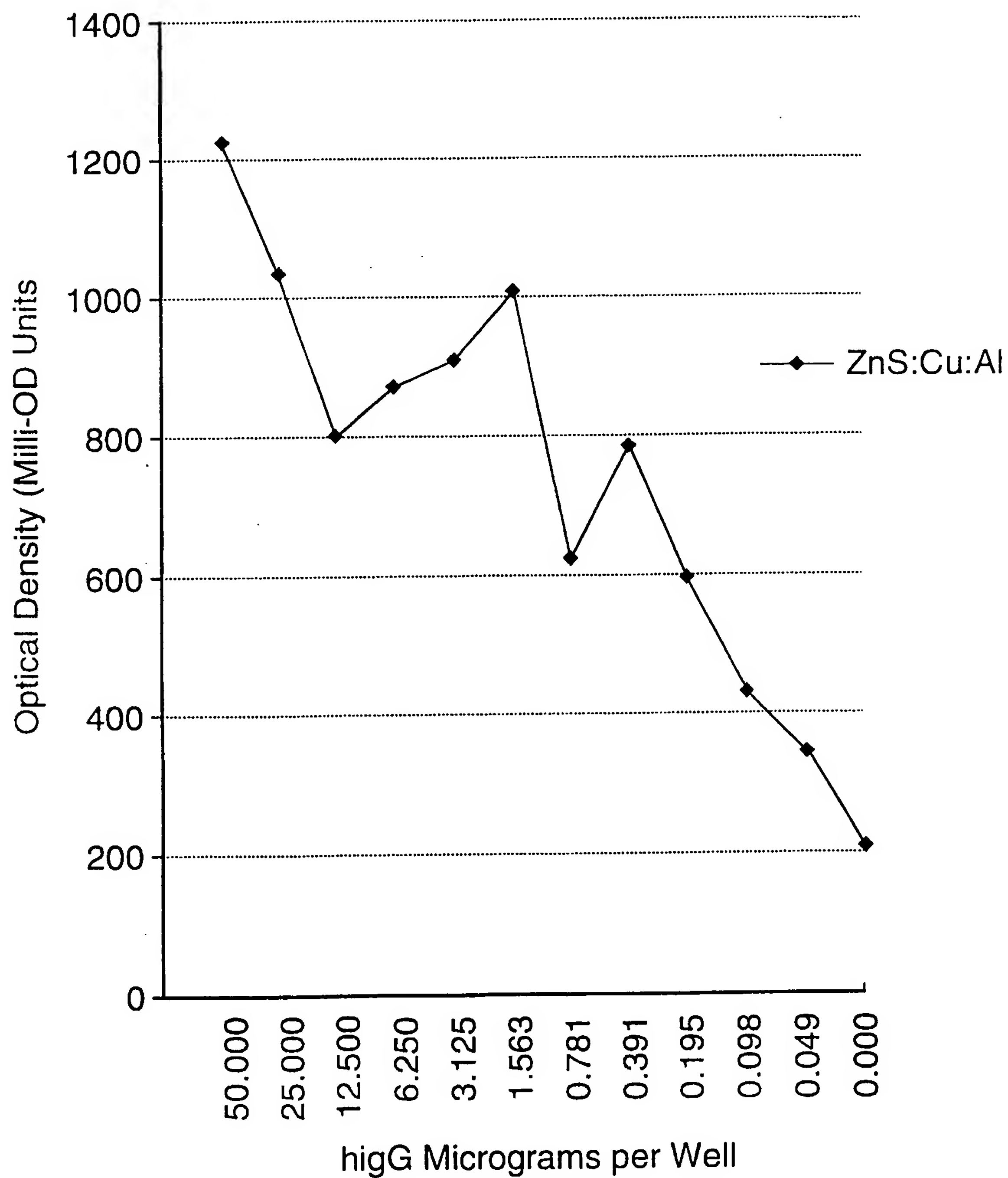


FIG.20

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 97/00828

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/543 G01N33/58 G01N27/327 G01N27/22 G01N21/64  
C12N7/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 87 03095 A (THE JOHN HOPKINS UNIVERSITY/APPLIED PHYSICS LABORATORY) 21 May 1987 see the whole document & US 4 822 566 A cited in the application ---	1-55
A	US 4 769 121 A (A. L. NEWMAN.) 6 September 1988 see the whole document ---	1-55
A	WO 88 08528 A (BIOTRONIC SYSTEMS CORPORATION) 3 November 1988 see the whole document ---	1-55
A	WO 89 11649 A (WOLLONGONG UNIADVICE LIMITED) 30 November 1989 see the whole document -----	1-55

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 97/00828

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WO 8703095 A	21-05-87	CA 1259374 A EP 0245477 A JP 63501446 T US 4822566 A	12-09-89 19-11-87 02-06-88 18-04-89
US 4769121 A	06-09-88	EP 0358693 A WO 8808461 A	21-03-90 03-11-88
WO 8808528 A	03-11-88	US 5114674 A EP 0358706 A	19-05-92 21-03-90
WO 8911649 A	30-11-89	NONE	



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>G01N 33/543, 33/58, 27/327, 27/22, 21/64, C12N 7/00, C12Q 1/68</b>		A1	(11) International Publication Number: <b>WO 98/21587</b> (43) International Publication Date: 22 May 1998 (22.05.98)
(21) International Application Number: PCT/CA97/00828 (22) International Filing Date: 7 November 1997 (07.11.97) (30) Priority Data: 08/746,420 8 November 1996 (08.11.96) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/746,420 (CIP) Filed on 8 November 1996 (08.11.96) (71) Applicant (for all designated States except US): NoAB IMMUNOASSAY INC. [CA/CA]; Unit #1, 50 Valleywood Drive, Markham, Ontario L3R 6E9 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): EWART, Thomas, G. [CA/CA]; 110 McClure Drive, King City, Ontario L7B 1B9 (CA). BOGLE, Gavin, T. [CA/CA]; Suite 406, 24 Wellesley Street, West, Toronto, Ontario M4Y 1G1 (CA). (74) Agent: WOODLEY, John, H.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published With international search report.
(54) Title: NANOPARTICLES BIOSENSOR			
(57) Abstract <p>Biosensor technology based on the labelling entities having particle reporters provides cost competitive readily manufactured assay devices. Submicron particles of uniform dimension in metals, polymers, glasses, ceramics and biological structures such as phages are used as the labelling entities. Such reporter particles greatly increase the sensitivity and accuracy, and provide a variety of assay techniques for determining analyte presence in a sample. The particles may have dielectric, paramagnetic and/or phosphorescent properties; such particles are particularly useful in a variety of competition type assays. Novel phosphor and phage particles are provided for use as unique labelling entities.</p>			



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## Rita Smolinsky

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**Sent:** Wednesday, August 09, 2006 8:24 AM  
**To:** 'marcpat@agcunhaferreira.pt'  
**Subject:** Trademark Department

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Mark: RIGHT GUARD  
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Our File: 12760-027002

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